Occupational kidney disease among young populations in northwest Nicaragua

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Abstract

Chronic kidney disease of undetermined aetiology (CKDu) is mainly responsible for the deaths of young males in agricultural lowland regions of Central America. The aim of this thesis was to advance the understanding of the causes of CKDu by conducting several different epidemiological research studies.

The systematic review identified a number of cross-sectional studies and occupational cohort studies with limited follow-up periods, but the findings were inconclusive regarding the causes of CKDu.

The cohort study described in this PhD thesis is the first community-based cohort study in the region to evaluate the natural history of disease in apparently healthy people aged 18-30 years. It collected information about a wide range of exposure conditions with a questionnaire, biological samples, and water samples. There was an unparalleled, asymptomatic and very rapid decline in renal function among 10% of males and 3.5% of females who had normal renal function at baseline. Meanwhile, the group displaying established renal dysfunction (mean estimated glomerular filtration rate, eGFR: 58 mL/min/1.73 m^2) at baseline showed a slower subsequent decline in kidney function (3.6 mL/min/1.73 m^2/year). A rapid decrease in eGFR was associated with outdoor work, agricultural work, and a lack of shade during work breaks.

The nested case-control study measured eGFR, and urinary neutrophil gelatinase-associated lipocalin (uNGAL) at baseline. After adjusting for eGFR,

uNGAL did not improve prediction a rapid decline in kidney function among individuals who initially presented a normal eGFR.

This is the first study to show that in northwest Nicaragua, 10% of the male and 3.5% of the female population aged 18-30 years showed a very strong decline in kidney function. Larger community-based and occupational longitudinal studies with more detailed analyses of exposure data are needed to identify the cause(s) of CKDu.

Table of Contents

List of tables

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

List of figures

Chapter 2

Chapter 4

Chapter 5

Chapter 6

Abbreviations

AKI	Acute kidney injury
A	Albumin
As	Arsenic
AA	Aristolochic acid
AUC	Area under the curve
ADPKD	Autosomal dominant polycystic kidney disease
BUN	Blood urea nitrogen
BEN	Balkan nephropathy
BMC	BioMed Central
BMI	Body mass index
BMJ	British Medical Journal
CKD	Chronic kidney disease
CKDu	Chronic kidney disease of unknown aetiology
CI	Confidence intervals
Cd	Cadmium
Cr	Chromium
CISTA	Centro de Investigación en Salud, Trabajo y Ambiente (Research Centre on Health, Work and Environment)
CKJ	Clinical Kidney Journal
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CRIC	Chronic renal insufficiency cohort study
DKF	Decreased kidney function
eGFR	Estimated glomerular filtration rate
ESRD	End stage renal disease

GMM	Growth mixture modelling
GFR	Glomerular filtration rate
Hg	Mercury
HIV	Human immunodeficiency virus
HT	Hypertension
HR	Heart rate
IL-18	Interleukin 18
IDMS	Isotope dilution mass-spectrometry
INSS	Instituto Nicaragüense de Seguridad Social (Nicaraguan Social Security Institute)
JASN	Journal of the American Society of Nephrology
KIM-1	Kidney injury molecule-1
KDIGO	Kidney Disease Improving Global Outcomes
LMICs	Low and middle income countries
Li	Lithium
MeN	Mesoamerican nephropathy
MAT	Microscopic agglutination test
MINSA	Ministerio de Salud (Ministry of health)
MDRD	Modification of diet in renal disease
NSAIDs	Non-steroidal anti-inflammatory drugs
NAG	N-acetyl-β-D-glucosaminidase
NGAL	Neutrophil gelatinase-associated lipocalin
OR	Odds ratio
OSHA	Occupational Safety Health Administration
OPs	Organophosphates

OTA	Ochratoxin A
Pb	Lead
PORs	Prevalence odds ratios
ROC	Receiver operating characteristic
RRT	Renal replacement therapy
Scr	Serum creatinine
Scys	Serum cystatin C
SD	Standard deviation
US	United States
UK	United Kingdom
uNGAL	Urinary neutrophil gelatinase-associated lipocalin
UACR	Urinary albumin-creatinine ratio
UTI	Urinary tract infection
WBGT	Wet Bulb Globe Temperature
WE	Worker health and efficiency programme

Chapter 1. General background

1.1 Chronic kidney disease

Chronic kidney disease (CKD) is an abnormality in kidney function or structure that is present for three or more months.[1] Kidney function is usually measured by calculating the estimated glomerular filtration rate (eGFR) from biomarkers (usually serum creatinine) measured in the blood.[2] A moderate to severe kidney function impairment (stage 3-5 CKD) is defined as an eGFR of <60 mL/min/1.73 m^2 for at least 3 months.[1] In countries such as the US and the UK, the main known risk factors for CKD are ageing, diabetes, hypertension, obesity and glomerulonephritis.[1]

1.1.1 Estimated glomerular filtration rate (eGFR)

Several methods have been used for measuring glomerular filtration rate (GFR). For example, Inulin, Iohexol or Iothalamate clearance have been frequently used for measurements of GFR and both methods perform very well. However, these methods are difficult to implement in field research or clinical practice because they are invasive, challenging to implement in field and carry risks some minor adverse effects, etc.

Currently, different equations have been developed to estimate the glomerular filtration rate based on serum creatinine. The most widely used equations are Cockcroft-Gault,[3] the Modification of Diet in Renal Disease (MDRD)[4-6] and recently the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[7] However, creatinine-based estimates appear to be affected by different sources of variation such as diet, body mass index (BMI), ethnicity, medications use, etc.[8]

Therefore, the international guidelines (Kidney Disease Improving Global Outcomes (KDIGO)) in 2012 has recommended the use of an additional marker-based method for eGFR calculation which include cystatin C. Also, equations use a combination of cystatin C and creatinine which offer an improved precision and accuracy if compared to creatinine-based estimates alone using CKD-EPI, particularly at higher kidney function (eGFR: >60 mL/min/1.73m^2).[1, 2, 7] However, this equation has not been validated for Latin-American population yet.

Table 1: Chronic kidney disease epidemiology collaboration (CKD-EPI) equation for estimating the GFR based on creatinine, cystatin c and creatinine and cystatin c[2, 7]

Formula and Sex	Serum creatinine levels (mg/dL)	Serum Cystatin C levels (mg/liter)	Formula for estimating GFR
CKD-EPI formula for Scr			
Male	≤0.9		*eGFR: 141 x (Scr/0.9)$^{-0.411}$ x 0.993^{Age} [x 1.159 if black]*
	>0.9		*eGFR: 141 x (Scr/0.9)$^{-1.209}$ x 0.993^{Age} [x 1.159 if black]*
Female	≤0.7		*eGFR: 144 x (Scr/0.7)$^{-0.329}$ x 0.993^{Age} [x 1.159 if black]*
	>0.7		*eGFR: 141 x (Scr/0.9)$^{-1.209}$ x 0.993^{Age} [x 1.159 if black]*
CKD-EPI formula for Scys			
Male or female		≤0.8	*eGFR: 133 x (Scys/0.8)$^{-0.499}$ x 0.996^{Age} [x 0.932 if black]*
Male or female		>0.8	*eGFR: 133 x (Scys/0.8)$^{-1.328}$ x 0.996^{Age} [x 0.932 if black]*
CKD-EPI formula for Scr and Scys			
Male	≤0.9	≤0.8	*eGFR: 135 x (Scr/0.9)$^{-0.207}$ x (Scys/0.8)$^{-0.375}$ x 0.995^{Age} [x 1.08 if black]*
		>0.8	*eGFR: 135 x (Scr/0.9)$^{-0.207}$ x (Scys/0.8)$^{-0.711}$ x 0.995^{Age} [x 1.08 if black]*
	>0.9	≤0.8	*eGFR: 135 x (Scr/0.9)$^{-0.601}$ x (Scys/0.8)$^{-0.375}$ x 0.995^{Age} [x 1.08 if black]*
		>0.8	*eGFR: 135 x (Scr/0.9)$^{-0.601}$ x (Scys/0.8)$^{-0.711}$ x 0.995^{Age} [x 1.08 if black]*
Female	≤0.9	≤0.8	*eGFR: 130 x (Scr/0.7)$^{-0.248}$ x (Scys/0.8)$^{-0.375}$ x 0.995^{Age} [x 1.08 if black]*
		>0.8	*eGFR: 130 x (Scr/0.7)$^{-0.248}$ x (Scys/0.8)$^{-0.711}$ x 0.995^{Age} [x 1.08 if black]*
	>0.9	≤0.8	*eGFR: 130 x (Scr/0.7)$^{-0.601}$ x (Scys/0.8)$^{-0.375}$ x 0.995^{Age} [x 1.08 if black]*
		>0.8	*eGFR: 130 x (Scr/0.7)$^{-0.601}$ x (Scys/0.8)$^{-0.711}$ x 0.995^{Age} [x 1.08 if black]*

Abbreviations: eGFR: estimated glomerular filtration rate; CKD-EPI: chronic kidney disease epidemiology collaboration equation; Scr: serum creatinine, Scys: serum cystatin c.

1.1.2 Classification of CKD

Central America has adopted the classification of CKD developed by the United States National Kidney Foundation as part of KDIGO 2012 criteria.[1] CKD is diagnosed by using both blood and urine markers. It can also be diagnosed using imaging or biopsy, but these methods are generally not accessible to much of the population in low- and middle-income countries (LMICs) in Mesoamerica. An eGFR is calculated using age, sex and biomarkers from the blood (serum creatinine or cystatin c). Additionally, the amount of protein loss in the urine (usually albumin) is usually quantified as the albumin/creatinine ratio. Two measurements recorded at least three months apart are required to confirm chronicity. Based on the two measurements, CKD is diagnosed and classified into five stages (see Table 1), including the mild stages (1-2) and moderate to severe stages (3-5).[1]

Table 2: KDIGO 2012 classification of CKD prognosis by eGFR categories and albuminuria levels[1]

				Albuminuria categories		
				A1	**A2**	**A3**
	Stages of CKD	**eGFR categories**	**Clinical interpretation**	<30 mg/g (normal to mildly increased)	30-300 mg/g (Moderately increased)	>300 mg/g (Severely increased)
eGFR categories (mL/min/1.73 m^2)	G1	>90	Normal			
	G2	60 – 89	Mildly decreased			
	G3a	45 – 59	Mildly to moderately decreased			
	G3b	30 – 44	Moderately to severely decreased			
	G4	15 – 29	Severely decreased			
	G5	<15	Kidney failure			

Abbreviations: eGFR: estimated glomerular filtration rate; CKD: chronic kidney disease.

Green: low risk; yellow: moderately increased risk; orange: high risk; and red: very high risk.

CKD is often defined solely on the basis of an eGFR<60 mL/min/1.73m^2. However, based on the results from a consortium of international studies performed primarily in developed nations (the CKD Prognosis Consortium), proteinuric kidney disease can exhibit the same risks of poor outcomes (cardiovascular diseases, death and complete renal failure), even if the eGFR is initially not low.[9, 10]

1.2 Acute kidney injury

Acute kidney injury (AKI) is defined as a sudden kidney impairment due to an increase in serum creatinine (Scr) levels of ≥0.3 mg/dL within 48 hours; an increase in Scr levels to ≥1.5 times the baseline value, which is known or presumed to have occurred within the previous 7 days; or a poor urine volume of 0.05 mL/kg/h for 6 hours (See Table 2).[11]

Table 3: Criteria for the diagnosis and classification of AKI[11]

Stage	Serum creatinine level	Urine output
1	1.5 – 1.9 times the baseline value or ≥0.3 mg/dL (≥26.5 µmol/L) increase within 48 hours	<0.5 mL/kg/h for 6-12 hours
2	2.0 – 2.9 times the baseline value	<0.5 mL/kg/h for ≥12 hours
3	3.0 times the baseline value or an increase in serum creatinine levels to ≥4.9 mg/dL (≥353.6 µmol/L) or initiation of renal replacement therapy or in patients <18 years, a decrease in eGFR to <35 mL/min/1.73 m^2	<0.3 mL/kg/h for ≥24 hours or anuria for ≥12 hours

1.3 Chronic kidney disease of unknown aetiology

Chronic kidney disease of unknown aetiology (CKDu), also known as Mesoamerican nephropathy (MeN) when it occurs in Latin America, has emerged over the last two decades and represents a major public health problem in rural communities in Mesoamerica.[12-18] This form of CKD is not associated with conventional CKD risk factors, such as diabetes, hypertension, glomerulonephritis and obesity.[15, 18-21] According to epidemiological studies, this disease mainly affects (but is not restricted to) young agricultural workers who live on the Pacific coast and typically work in occupations requiring high intensity physical activity in hot environments. Clinical and laboratory studies have shown asymptomatic increases in serum creatinine levels and normal blood pressure, no or only low-grade proteinuria, hyponatremia, hypokalaemia, and hyperuricaemia,[15, 18, 21-27] and both tubulointerstitial and glomerular damage upon biopsy.[28, 29]

1.3.1 Prevalence of CKDu

The prevalence of CKDu (as estimated by a SINGLE measure of eGFR<60 mL/min/1.73 m^2) is highly variable, depending on the geographic area. It predominantly affects rural populations, with a prevalence ranging from 13.4% to 26%, compared to much lower levels in urban populations (0.0% to 9.1%), where the highest reported prevalence is approximately half the rural estimates.[15, 18-21, 30] Prevalence appears to be lower at higher altitudes (1.2 to 7.5%) than at sea level (0% to 18.5%).[15, 18, 31]

Differences in prevalence with age, sex and certain occupations have also been observed. Relatively young age groups, e.g., individuals aged 18-40 years, appear to be particularly affected, with a prevalence rate ranging from 8.1% to

38.5%. Males are more frequently affected, with the highest estimates ranging from 13.8% to 42%, compared to females with a range of 5.8% to 22%; the average male:female ratio is 2:1.[15, 18-21, 30, 32] Most affected individuals have been rural sugarcane workers, with the highest prevalence (13% to 44% for males),[15, 18, 20, 21, 24] followed by miners (19%), and male banana/sugarcane workers (17%).[15] Construction workers are also affected, with a prevalence of 9%.[24] Individuals with other occupations seem to be less affected, e.g., coffee workers and service workers, with prevalences that do not exceed 7.5%.[15, 18, 31]

A recent publication reported excess mortality due to CKD in the last 16 years (1997-2013) in Central America. The age-standardized mortality rate increased in Nicaragua from 23.9 deaths per 100 000 population in 1997 to 36.7 deaths per 100 000 population in 2013; in El Salvador, mortality increased from 18.7 to 47.4 deaths per 100 000 population in the same period. These rates are approximately nine and twelve times higher in El Salvador and Nicaragua than in other countries in Central America.[33]

1.3.2 Potential causes of CKDu

A variety of hypotheses regarding the aetiology and factors contributing to CKDu in Mesoamerica have been proposed. However, no clear causal mechanisms have been identified. The leading hypotheses are occupational heat stress, recurrent dehydration, pesticide exposure, environmental toxin (heavy metal) exposure, and infectious diseases. The non-occupational hypotheses include self-medication with NSAIDs, homemade alcohol consumption, and genetic predisposition.[12, 13]

1.3.2.1 Heat stress/dehydration

Agricultural and non-agricultural workers in Mesoamerica work under extremely hot conditions with intense physical demands for long hours each day. Given these conditions, volume depletion due to recurrent acute or severe dehydration episodes in sugarcane workers has been proposed to cause subclinical acute kidney injury (AKI) due to reduced renal blood flow, leading to ischaemia.[34-39]

To date, several studies have explored the role of heat-stress/cyclical dehydration and decreased kidney function. Environmental studies of sugarcane workers in Costa Rica and El Salvador have shown that at approximately 9:00 a.m. heat/humidity exceeds the recommendation (WBGT>27ºC) of the Occupational Safety Health Administration (OSHA).[22, 40, 41] The most common symptoms reported by sugarcane workers have been headache (71%), tachycardia (46%), muscle cramps in the arms/legs (39%), dysuria (36%), fever (28%), and nausea (26%).[42] However, scientific evidence is inconclusive as to whether these symptoms are pathognomonic of heat stress, either among sugarcane workers, or in workers in other occupations around the world.

Cross-shift studies among harvesters in El Salvador have shown that sugarcane workers suffer from an asymptomatic increase in Scr, uric acid, urea nitrogen, and urine specific gravity levels, as well decreased serum potassium and sodium levels after their shifts.[22] The post-shift (work time of 4 hours) Scr levels increased 10%. This change might occur due to a substantial loss of body water and salt, resulting in vasoconstriction secondary to dehydration.

However, the absence of differences in haematocrit, serum osmolarity and a decrease in urinary neutrophil gelatinase-associated lipocalin (uNGAL) levels after the shift are evidence arguing against a recurrent dehydration-AKI hypothesis.[22] Similar findings were reported among burnt sugarcane harvesters in Brazil, where 5 out of 28 (17.8%) workers experienced an increase in serum creatinine levels greater than 0.3 mg/dL after their work shifts (compatible with AKI), as well as increases in serum creatine, phosphokinase and specific urinary gravity levels after the work shift.[43] In addition, a study among agricultural workers from California reported an incidence of AKI (increase in serum creatinine levels ≥0.3 mg/dL post-shift) of 12% (35/283 workers) among males and females. Of these only 3 workers suffered heat strain. The main risk factors associated with AKI were an experience of heat strain (OR: 1.35, 95% CI 1.04-1.74) and piece rate work (OR: 4.24, 95% CI 1.56-11.52).[44]

Two prospective occupational studies with short-term follow-up periods examined the changes in renal biomarkers among cane workers in Nicaragua.[23, 45] The first study reported significant clinical changes in some kidney biomarkers, such as a 20% increase in the serum creatinine level, a four-fold increase in uNGAL levels, a 41% increase in BUN levels, and an approximately four-fold increase in urinary uric acid levels across the harvest period. Furthermore, participants experienced changes in weight and electrolyte disturbances (hyponatremia and hypokalaemia), suggesting a volume depletion due to dehydration.[23] The authors of the study proposed the crystalluria theory, which is based on the following assumptions: work under hot conditions with

poor hydration can produce volume depletion with subsequent salt loss, an increase in serum osmolarity and a subclinical muscular injury that can induce high uric acid levels with a low urine pH and crystalluria, followed by tubular injury.[46-48] However, uric acid crystals have not been observed in renal tissues from sugarcane workers in El Salvador and Nicaragua.[28, 29, 49]

The second study observed a change in kidney function among sugarcane workers across the harvest period (6 months). The job categories that experienced a significant change in eGFR were seed cutters -8.6 mL/min/1.73 m^2 (95% CI: -16.7 to -0.5) and water irrigators -7.4 mL/min/1.73 m^2 (95% CI: -12.6 to -2.1). However, the eGFR changes among cane cutters (-5.0 mL/min/1.73 m^2; 95% CI: -10.5 to 0.6) and pesticide applicators (-3.8 mL/min/1.73 m^2; 95% CI: -9.9 to 2.3) were not statistically significant, although both jobs are considered to have a very heavy work load and are performed in a hot environment. Moreover, no changes in eGFR were observed among factory workers (3.2 mL/min/1.73 m^2).[45] In addition, cane cutters and water irrigators experienced approximately two-fold increases in the urinary levels of NGAL and IL-18. Meanwhile, the NAG level increased in almost all job categories. Only 5% of cane workers displayed an increase in the urine albumin-creatinine ratio (UACR) exceeding 30 mg/g.[26]

Recently, a small cross-sectional study and a subsequent follow-up study of a group of cane workers with decreased kidney function (DKF) at late harvest reported that the prevalence of DKF was 10.4% (34/326) among young sugarcane workers across the harvest period (median Scr level of 1.64 mg/dL).

DKF was most common among cane cutters (19%) than among other cane jobs (2.9%). Twenty-nine workers diagnosed with DKF showed improvements in Scr levels of 0.39 mg/dL (median Scr level of 1.64 mg/dL to 1.25 mg/dL) during the first 6 months of follow-up. However, twenty-five of the 29 participants that were followed 12 months after the baseline experienced a non-significant decrease in Scr levels of 0.37 mg/dL compared to the previous study visit. In addition, thirty-eight percent workers (11/29) experienced a dramatic decrease in eGFR (>30%) during the course of the study. Of these workers, ten workers had an eGFR <60 mL/min/1.73 m^2.[50] Based on these results, a group of workers with chronic CKDu were followed and were diagnosed with AKI simply because they crossed the threshold of 0.3 mg/dL during the harvest season.

To date, the results from epidemiological studies investigating the relationships between heat stress exposure, AKI and subsequent CKDu remain inconclusive. This lack of conclusive data may be due to insufficiently robust heat exposure assessments, a non-optimal study design or inadequately sensitive biomarkers to accurately quantify the exposure and effects.[22, 46, 51] To date, the strongest evidence supporting this proposed mechanism of CKDu has been obtained from animal models.[48, 52] Thus, more studies in humans are urgently needed to test these suggested mechanisms, to explore other exposure methods and potential mechanisms and to study other occupations with similar characteristics around the globe.

1.3.2.2 Agrochemical exposure

Pesticides have been used intensively and extensively in agriculture to protect crops from insects and other pests in low-middle-income countries (LMICs).[53]

These chemicals are applied at the ground level (manual backpack sprayers or hydraulic sprayers) and through aerial spraying; thus, agricultural workers, their families and the communities surrounding the plantations are directly or indirectly exposed to pesticides.[53] Short- or long-term exposure to pesticides can induce acute or chronic health effects on various organs, including the gastrointestinal tract, respiratory system, cardiovascular system, nervous system and kidneys.[54-57]

Organophosphates (OPs) and organochloride pesticides (e.g., glyphosate, 2,4-dichlorophenoxyacetic acid, chlorpyrifos and other OPs) have been proposed as leading or contributing factors to CKDu in Mesoamerica and other countries.[20, 58-64] The assumed nephrotoxic effects of these agents are based on case reports of workers who developed AKI after the intentional ingestion of dimpylate and methamidophos,[65, 66] but little experimental data has suggested a chronic renal tubular cytotoxicity.[67, 68] Eleven epidemiological studies have examined the associations between pesticide exposure and CKDu in Mesoamerica, all of which were based on binary variables (yes/no). Only two studies reported a positive correlation between agrochemical exposure and CKDu; the first study showed a positive correlation between self-reported pesticide inhalation and CKD (OR: 3.14, 95% CI: 1.12-8.78), but not other exposure parameters, such as short- or long-term exposure, exposure frequency, mixing or applying these chemicals.[21] The second study reported a correlation between self-reported pesticide exposure, regardless of the absorption pathway, with CKD ($p<0.0001$) in a univariate analysis, but not in the

model adjusted for age, gender, body mass index (BMI), hypertension, diabetes, and a family history of CKD.[69]

In contrast, a study conducted in a coffee-farming agricultural community found that pesticide exposure (applying, mixing or both) was not associated with decreased kidney function among farmers.[31] Similar findings were reported by Laws, et al., where pesticide applicators in the sugarcane industry did not experience significant changes in eGFR or uNGAL levels compared to factory workers throughout the harvest season.[45] Thus, uNGAL may not be the appropriate biomarker to detect damage caused by pesticide exposure.

Recently, a systematic review was conducted investigating the association between pesticide exposure and CKD. Twenty-one epidemiological articles were included in the final analysis, and 62% of these studies reported positive correlations between pesticide exposure and CKD. However, these studies had methodological limitations due to incomplete adjustments for confounding factors, selection bias and exposure measurements, and these aspects affected the study quality. Thus, the evidence for a role for pesticide exposure in CKD remains inconclusive.[62]

1.3.2.3 Heavy metal exposure

Heavy metals that have been associated with kidney damage include lead (Pb), cadmium (Cd), chromium (Cr), arsenic (As), mercury (Hg) and lithium (Li). Recently, Tsai, et al. examined the longitudinal associations between cadmium (Cd), chromium (Cr) and lead (Pb) exposure and changes in kidney function among Taiwanese adults aged 19-84 years from the National Nutrition and

Health Survey from 2005-2008. A decrease in eGFR was associated with high levels of Cr (-5.9 mL/min/1.73 m^2; 95% CI: -9.7 to -2.2) and Pb (-6.6 mL/min/1.73 m^2; 95% CI: -9.7 to -3.5), but not Cd (2.0 mL/min/1.73 m^2; 95% CI: -3.2 to 7.2), and the trend towards a reduced eGFR was similar among males and females. However, when the analysis was adjusted for potential confounding factors (age, education, BMI, smoking, nutrient intake of sodium and urinary volume) and stratified by tertiles of urinary Cd, levels of urinary Cr (-12.6 mL/min/1.73 m^2; 95% CI: -20.4 to -4.9) and urinary Pb (-11.2 mL/min/1.73 m^2; 95% CI: -17.0 to -5.4) were both associated with decline in kidney function only in the highest Cd tertile (>1.02 µg/L). This study suggests an interaction between levels of Cd and exposure to Pb and Cr in mediating any renal effects.[70]

Similar findings were reported in a cohort study by Harari, et al. (2018),[71] where an increased Pb level (>29 µg/L) was associated with lower eGFR based on serum creatinine and cystatin c levels (-2.6 mL/min/1.73 m^2; 95% CI: -4.0 to -1.2) or only creatinine levels (-2.9 mL/min/1.73 m^2; 95% CI: -4.3 to -1.5). High lead levels (>29 µg/L) were still associated with a decrease in eGFR over a 21-year follow-up period after adjusting for an age >58 years, sex, hypertension, a history of diabetes, waist circumference (>82 cm), smoking and alcohol consumption.[71] These studies reported positive correlations between Pb and Cr exposure with kidney function,[70, 71] and these metals have been shown to induce chronic tubulointerstitial damage and nephrosclerosis due to intranuclear lead inclusions in studies of tubular epithelial cells in vitro.[72]

Cadmium poisoning can lead to or contribute to renal dysfunction due to chronic occupational exposure, contaminated food or an unhealthy lifestyle (smoking). The effects of Cd on the kidney include impaired proximal tubular cell reabsorption, which results in increased urinary excretion of tubular proteins.[73] Renal damage is characterized by tubular atrophy, chronic glomerular damage, and interstitial fibrosis.[74]

To date, little evidence is available about the potential role of arsenic (As) in kidney disease. However, As plays an important role in oxidative stress and inflammation. These two possible mechanisms may drive endothelial dysfunction and subsequent kidney damage. In addition, long-term exposure to inorganic As in drinking water (>100 µg/L) was reported to be associated with kidney damage among a Taiwanese population.[75] Additionally, urinary levels of four heavy metals (As, Cd, Hg and Pb) were measured in a renal biopsy study in Nicaragua.[28] All of these metals were within the permissible limits for heavy metals in urine.[28] In summary, knowledge about the roles of heavy metals in the pathogenesis of CKDu is still lacking.

1.3.2.4 Infectious diseases

The most well-known infectious diseases associated with CKD are human immunodeficiency virus (HIV), hepatitis B and C virus. However, a list of zoonotic diseases has been proposed to induce acute kidney injury (AKI), including dengue, malaria, leptospirosis, and hanta virus. Similar pathogens have therefore have been suggested to cause CKD in patients with severe infections.[76, 77] Furthermore, organisms such as *Leptospira* have been

proposed as a cause of CKDu in hotspots in Mesoamerica, Sri Lanka, India, and other countries, because workers at risk of the disease (sugarcane workers, miners, rice workers, and agriculture workers) are also at high risk of exposure to water sources contaminated by infected animals.[78-80]

Several *Leptospira* outbreaks have been reported since 1995 due to natural disasters (tropical storms and hurricane) that have hit Nicaragua. The departments with the highest cumulative incidence rate of leptospirosis were León with 36.03/10,000 populations (522 cases), Chinandega with 36.03/10,000 populations (305 cases) and Managua 7.60/10,000 populations (175 cases) from 2004-2010.[81] Based on these data, a research group reviewed the leptospiral serology amongst a number of different occupations and found high rates of *Leptospira* infections among prawn workers (109/1000 workers), water irrigation and drainage workers (62/1000 workers) and cane collectors and cane cutters (36/1000 workers); however, the authors did not evaluate kidney function.[79]

Riefkohl A, et al. (2018), reported a high *Leptospira* seropositivity among Nicaraguan cane cutters (59%), water irrigators and seeders (37%). However, this high seropositivity was not associated with impaired kidney function in individuals with normal kidney function at pre- and post-harvest assessments. When using a linear regression analysis to compare sugarcane applicants with renal dysfunction at the pre-harvest assessment and *Leptospira* seropositivity, a suggestive inverse correlation was observed (eGFR mean difference: -10.08, 95% CI: -24.12 to 3.96), but not for NGAL (mean difference: -1.59, 95% CI: 0.81

to 3.11), IL-18 (mean difference: 0.84, 95% CI: 0.42 to 1.70), or NAG (mean difference: 1.44, 95% CI: 0.85 to 2.43), because the study was underpowered.[78] Thus, the evidence supporting a role for *Leptospira* infection in the aetiology of MeN remains limited.

Other studies have examined the association between *Leptospira* exposure and kidney disease in East Asia. A community-based survey recruited 3045 participants in Taiwan, with a subsequent follow-up of over two years to evaluate the risk of CKD in patients with leptospirosis.[82] The prevalence of leptospirosis was 33% in this population and 49% among males. A lower leptospirosis prevalence was found among patients with stage 3-5 CKD (14%) compared to patients with all stages of CKD (22%). The adjusted linear regression model showed that a positive reaction for *Leptospira* antibodies was strongly correlated with lower eGFR (-3.04 mL/min/1.73 m^2, 95% CI: -4.15 to -1.93), and this correlation remained positive when the analysis was stratified by diabetes mellitus status. At the end of the follow-up period (2009-2011), subjects with a high titre of the *Leptospira* antibody (MAT titre >400) had a lower eGFR (-13 mL/min/1.73 m^2) and a higher KIM-1/creatinine ratio than subjects with a MAT titre 100-200 and MAT-negative subjects. Thus, chronic leptospirosis (MAT titre >400) might induce CKD in areas where *Leptospira* is endemic.[82]

Dengue fever can induce AKI, nephrotic syndrome, glomerulopathy and other tubular abnormalities due to direct damage by immune complexes composed of antigens and antiviral antibodies. The prevalence of AKI secondary to dengue

fever ranges from 3.3 to 14.2% among infected adults.[83-85] A prospective cohort study evaluated the renal outcomes following AKI in patients hospitalized with dengue. The incidence of AKI was 13.7% (72/526 patients) and 9 of 10 patients (93%) had AKI on the first day of hospital admission; 7% were admitted to the hospital without AKI and were later diagnosed with AKI. Of these patients, 19 (26.8%) showed a full renal recovery at hospital discharge and the AKI of thirty-five patients did not recover at the time of discharge. At the end of the follow-up period (12 weeks), only eight patients (11.2%) did not exhibit a restoration of kidney function. Finally, the risk factors associated with a poor renal outcome (<60 mL/min/1.73 m^2) were sex (female), older patients, diabetes mellitus, the use of NSAIDs, a secondary infection, and no recovery upon discharge.[86] Because CKD is defined as a persistent loss of kidney function that is documented to persist for more than 3 months, the existing studies of kidney function in patients infected with dengue are insufficient to assess long-term outcomes of kidney function. Consequently, researchers have not currently determined whether dengue is associated with CKD, although suggestive associations with AKI have been reported.

The scientific evidence regarding an association between chikungunya virus and AKI has not been well described to date. Recently, a case-control study among patients with chikungunya in Bangladesh reported that 10% (11/107 chikungunya cases) developed AKI (≥0.3 mg/dL increase in serum creatinine levels at hospital admission) during the course of this disease.[87] Similar findings were reported by Perti T, et al. (2016), who showed that the prevalence of AKI was 22% (33 out of 153 patients) among veterans diagnosed with

chikungunya who presented a high risk of hospitalization (RR, 1.64; 95% CI, 1.33–2.04). The other comorbidities and clinical findings contributing to hospital admission included CKD, congestive heart failure, diabetes, chronic lung disease, tachycardia, leucocytosis and a high level of hepatic transaminitis.[88] However, these two studies have several limitations. First, the results cannot be extrapolated to other populations because the studies were based on hospital records and the sample size was small. Second, elderly participants were recruited, which might increase the risk of complication during clinical course. Thus, chikungunya virus must be systematically investigated to determine whether infected individuals in the general population are at risk of developing AKI.

1.3.2.5 Nephrotoxic medications

Non-steroidal anti-inflammatory drugs (NSAIDs) are associated with AKI and with accelerating the progression of CKD.[89] These drugs decrease the volume of renal blood flow by reducing prostaglandin synthesis and inducing acute tubular injury.[89, 90] However, a case-control study by Perneger TV, et al. (1994) found that chronic paracetamol ingestion may be more nephrotoxic than NSAIDs (ibuprofen/diclofenac), as the authors identified a three-fold increase in the risk of progression to end stage renal disease (ESRD) in subjects with an annual intake of paracetamol ≥366 pills/year. However, a cumulative lifetime intake of NSAIDs ≥5000 pills/lifetime was associated with a nearly nine-fold increased risk of progression to ESRD.[91] However, although a number of studies have examined NSAID exposure, none have identified a positive correlation with CKDu.[92]

1.3.2.6 Phyto/mycotoxins

A number of researchers have speculated that phytotoxins or mycotoxins are possible causes of CKDu.[12, 13] Balkan nephropathy (BEN) is now thought to be caused by consuming wheat grain products contaminated with aristolochic acid (AA). This disease primarily affects people aged between 30 to 60 years who live in the rural areas of the Balkans and is characterized by chronic tubulointerstitial nephritis and a slow progression to ESRD. Patients with this disease display a high incidence of urothelial cancer after kidney damage occurred.[93-95] The *Aristolochia* species has been identified in Central America, but to date, increase rates of urinary tract malignancy have not been reported.[12, 76, 96, 97]

The mycotoxin Ochratoxin A (OTA) is mainly produced by two *Aspergillus* species (*A. ochraceus* and *A. niger*) and *Penicillium* species (*P. verrucosum* and *P. carbonarius*). This molecule causes a wide range of health effects due to its hepatotoxic, carcinogenic, neurotoxic and nephrotoxic properties. Humans are exposed through three different routes: dietary intake, dermal contact and inhalation. OTA-induced kidney damage is described as an enlargement of the tubular membrane, collagen inclusions, a diffuse density and size of brush border processes due to oxidative stress and a depletion of intracellular glutathione followed by apoptosis.[98-100] Although the histological findings are similar to MeN,[28, 29, 49] as is the case with AA, the increase in the mortality rate from CKDu has not been mirrored by increases in death caused by urothelial cancers, which are known to be caused by OTA. Thus, the current

epidemiological evidence does not support a possible role for OTA in pathogenesis of CKDu.

Finally, the above literature review demonstrated that there is an excess of CKDu in Mesoamerica and elsewhere. The causes of this disease are still not fully understood. Table 4 summarises the current knowledge about the most common risk factors for traditional and non-traditional CKD in Mesoamerica and South Asia.[1, 12, 13, 60, 61, 76, 101-103]

Table 4: Most common risk factors for traditional and non-traditional chronic kidney disease in different settings

Risk factors	***CKD in high income countries***	***CKD in Mesoamerica***	***CKD in Sri Lanka, and India***
Age	Older adults	Young and middle age adults	Young and middle age adults
Sex	CKD more common in women but ESRD more common in men	CKD and ESRD more common in men	CKD and ESRD more common in men
Ethnicity	Many causes of CKD more common in certain ethnicities (e.g. diabetic kidney disease in South Asians, proteinuric CKD in African-americans)	Hispanic	South Asian
Altitude	No association described	More common at low altitude	Described in the lowland dry zone
Socioeconomic status	More common in those of lower socioeconomic status	More common in those of lower socioeconomic status	More common in those of lower socioeconomic status
Family history of CKD	Common	Common	Common
Obesity	Common	Uncommon	Uncommon
Hypertension	Common	Uncommon*	Uncommon*
Diabetes mellitus	Common	Uncommon*	Uncommon*
Kidney stones	Well described	Uncommon	Uncommon
Smoking	Common	Uncommon	Uncommon
Alcohol consumption	Association described in some populations	Association described	Association described

Risk factors	***CKD in high income countries***	***CKD in Mesoamerica***	***CKD in Sri Lanka, and India***
Nephrotoxic medications (e.g. phenacetin)	Described in some regions	Rarely described	Rarely described
Nephrotoxins (Ochratoxin A, Aristolochic acid)	Described in some regions	Not systematically studied	Not systematically studied
Heavy metals (cadmium, lead, mercury, and arsenic)	Described in some regions	No replicated associations	No replicated associations
Infectious diseases (Leptospira, Hanta virus)	Not described	Not systematically studied	Not systematically studied
Low birth weight	Well described	Not systematically studied	Not systematically studied
Occupations	Not described	Agricultural workers (mainly sugarcane workers)	Farmers and paddy workers
Heat-stress	Not described	Common	Not systematically studied
Physical exertion	Not described	Common	Not systematically studied
Dehydration or volume depletion	Not described	Common	Not systematically studied
Agrochemicals exposure	Association at a population level	Not systematically studied	Not systematically studied

* Diabetes, hypertension and heavy proteinuria are often used as clinical exclusion for a diagnosis of CKDu but these risk factors may co-exist in some of those with disease.

1.4 Thesis rationale, hypothesis, aim and objectives

1.4.1 Thesis rationale

CKDu has emerged as an important public health problem in Mesoamerica (Nicaragua, El Salvador, Costa Rica, Guatemala and Southern México) and is a form of kidney disease that is not caused by the traditional risk factors for CKD, such as diabetes, hypertension, and obesity. This disease is estimated to be responsible for the death of more than 20,000 young adults in the last three decades, and this number continues to increase at an alarming rate.[16, 33, 104-106] The most frequently affected individuals are young sugarcane workers, subsistence workers, miners, and construction workers who are living on the Pacific coast of Nicaragua.[12-14, 104]

In addition, CKDu has social implications, because it affects the economically productive population, is a major cause of disability and limitations to generate income, increases out-of-pocket medical expenses, and requires other members in a given family to dedicate part of their time to care for the affected patient.[107] Even when health authorities have tried to respond, the actions are limited, such as a resource-consuming domestic peritoneal dialysis programme in affected areas, which is implemented in high poverty regions.

The causes and aetiology of this disease are not yet completely understood. Many hypotheses have been suggested, and the current leading theory is that the combination of repeated heat stress, dehydration and strenuous work in a hot environment is a predominant driving factor of disease development.

Environmental contaminants (heavy metals and pesticides), self-medication and infectious diseases may also contribute.[12, 13, 101, 108, 109]

A number of epidemiological studies have assessed the prevalence of CKDu among young agricultural workers and the general population who live at different altitudes in Mesoamerica. These studies have identified possible risk factors for CKDu, but none have been able to identify the cause(s) of this disease. This lack of causality is perhaps because more than 90% of the studies have employed a cross-sectional design, which has several limitations. These limitations include difficultly in determining whether the outcome followed exposure over time, a susceptibility to low response rate and misclassification due to recall bias and reverse causation. On the other hand, longitudinal studies may overcome some of these problems and allow researchers to confirm or reject the aforementioned hypotheses to help establish causality.

1.4.2 Hypothesis

The hypothesis underlying this work is that CKDu is caused by a combination of risk factors related to the local agricultural industry, such as recurrent heat exposure and/or exogenous toxins.

The work described in this thesis is part of a larger ongoing programme of work which aims to understand causation of CKDu using a range of global ongoing studies. Specific aims of this thesis are as outlined in section 1.4.3.

There are aspects of work not addressed in this thesis which are being done as part of the larger programme, for instance the cohort described in this thesis is being expanded at present to investigate further hypothesis of causation.

1.4.3 Aims and objectives

1.4.3.1 Aims of the thesis

The aim of this thesis was to advance the understanding of the causes of CKDu using a number of epidemiological research studies.

1.4.3.2 Specific objectives

1. To review the current knowledge and gaps in our understanding of the potential causes of CKDu in the Pacific coast of Central America.
2. To understand which risk factors are associated with the decline of eGFR among a healthy young population at risk of developing CKDu.
3. To determine if repeated routine creatinine tests combined with baseline urinary measurements of urinary neutrophil gelatinase-associated lipocalin (uNGAL) levels can identify the sub-group of individuals at risk of a future rapid decline in kidney function.

A systematic review was conducted to achieve the first objective (Paper I). Peer-reviewed scientific papers were collected from a variety of sources because epidemiological studies about CKDu have been published in many different journals.

A community-based prospective longitudinal study was conducted among a young, apparently healthy population in northwest Nicaragua to achieve the second objective. The study involved the collection of biological samples (blood, urine) and water samples, and the administration of a questionnaire that collected data on a variety of characteristics and exposure factors (sociodemographic data, current and past occupation, heat stress and pesticide exposure, lifestyle, self-medication, heat-stress symptoms, etc.) (Papers II and III). Paper II specifically discusses the rationale for conducting a community-based cohort study and describes the main findings obtained at the baseline study visit. Paper III investigated the natural history and factors associated with a decline in kidney function in a high-risk population.

A simple predictive score was created based on our existing community-based prospective cohort study to achieve the third objective. The purpose was to determine if repeated routine creatinine tests combined with baseline urinary measurements of urinary neutrophil gelatinase-associated lipocalin (uNGAL) levels can identify the subgroup of individuals at risk of a future rapid decline in kidney function (Paper IV). Table 3 summarizes the objectives and study design details.

Table 5: Thesis objectives and study designs

Objectives	Paper title	Study design	Population	Data sources	Exposure	Outcome
1. To review the current knowledge and gaps in our understanding of the potential causes of CKDu in the Pacific coast of Central America.	What do epidemiological studies tell us about chronic kidney disease of undetermined cause in Meso-America? A systematic review and meta-analysis	Systematic review	Adults and adolescents	PubMed EMBASE Web of Science	Heat stress, dehydration, pesticide exposure, NSAIDs, occupational exposure, environmental toxins and infectious diseases	CKDu
2. To understand which risk factors are associated with the decline of eGFR among a healthy young population at risk of developing CKDu.	Rationale, description and baseline findings of a community-based prospective cohort study of kidney function amongst the young rural population of northwest Nicaragua Decline in kidney function among apparently healthy young adults at risk of Mesoamerican nephropathy	Prospective cohort study	Young adults aged between 18 – 30 years without a diagnosis of CKD, diabetes or HT	Primary	Sociodemographic data, occupational, workplace conditions, environmental, lifestyle, and infectious diseases	Decline in eGFR over time
3. To determine if repeated routine creatinine tests combined with baseline measurements of urinary neutrophil gelatinase-associated lipocalin (uNGAL) levels can identify the sub-group of individuals at risk of a future rapid decline in kidney function.	Identification of young adults at risk of an accelerated loss of kidney function in an area affected by Mesoamerican nephropathy	Case-control study nested in a prospective cohort study	Young adults aged between 18 – 30 years without a diagnosis of CKD, diabetes or HT	Primary	Sociodemographic data, outdoor work and laboratory results (uNGAL, UACR)	Prediction decline of eGFR

Abbreviations: *CKDu: chronic kidney disease of unknown origin; eGFR: estimated glomerular filtration rate; CKD: chronic kidney disease; HT: hypertension; NSAIDs: non-steroidal anti-inflammatory drugs, uNGAL: urinary neutrophil gelatinase-associated lipocalin, UACR: urinary albumin-creatinine ratio.*

1.4.4 Thesis structure

This thesis includes 6 chapters that are structured into different sections: background, methods, the results and discussion. This first chapter provides information about the thesis rationale, hypothesis, aim and objectives. The specific objectives were addressed by a variety of study designs (Table 3). A systematic review and formal meta-analysis of the current knowledge of CKDu in Mesoamerica is presented in the second chapter to complete the background section (Objective 1).

The third chapter describes the methods, data sources, study populations and materials used in the community-based longitudinal study over the two-year period. It also presents the general results obtained at the baseline study visit (Objective 2). The fourth chapter investigates the natural history of and factors associated with the loss of kidney function in a high-risk population (Objective 2). The fifth chapter assesses whether repeated routine creatinine tests combined with baseline urinary measurements of urinary neutrophil gelatinase-associated lipocalin (uNGAL) levels can identify the sub-group of individuals at risk of a future rapid decline in kidney function among an apparently healthy young male population in Nicaragua (Objective 3).

Finally, the sixth chapter summarizes and discusses the main results of each study, describes the strengths and limitations of the thesis and considers the implications for clinical practice and future research on this topic.

Chapter 2. Systematic review of the current knowledge and gaps in the knowledge of chronic kidney disease of unknown origin.

2.1 Introduction to paper I

This paper was published in Clinical Kidney Journal (CKJ) and presents a systematic review and meta-analysis of the current epidemiological evidence and gaps in our knowledge of CKDu in Mesoamerica.

Twenty-five epidemiological studies were included in this analysis of risk factors for CKDu. Studies included participants of different ages who were living in diverse communities, mostly at sea level. Overall, the assessment of the quality of the epidemiological studies was medium. The quality of the occupational and community-based studies was affected by a substantial lack of follow-up data, potential for reverse causation, a lack of complete adjustment for confounding factors and the use of single measurements of serum creatinine to diagnose CKDu. The principal risk factors associated with CKDu were male sex, a family history of CKD, lowland altitude, and high water-intake. Also, sugarcane work appears to be associated with an elevated risk of CKDu in Mesoamerica. However, in this review, no positive correlations between pesticide exposure, NSAIDs, alcohol consumption, heat stress and CKDu were identified.

The study search strategies, inclusion and exclusion criteria, study quality assessments and funnel plots mentioned in the paper as supplementary material are included in this chapter.

2.2 Research paper cover sheet

RESEARCH PAPER COVER SHEET

PLEASE NOTE THAT A COVER SHEET MUST BE COMPLETED <u>FOR EACH</u> RESEARCH PAPER INCLUDED IN A THESIS.

SECTION A – Student Details

Student	Marvin Gonzalez-Quiroz
Principal Supervisor	Dorothea Nitsch
Thesis Title	Occupational kidney disease among young populations in northwest Nicaragua

<u>If the Research Paper has previously been published please complete Section B, if not please move to Section C</u>

<u>SECTION B – Paper already published</u>

Where was the work published?	Clinical Kidney Journal		
When was the work published?	2018		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	No		
Have you retained the copyright for the work?*	**Yes**	Was the work subject to academic peer review?	**Yes**

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<u>SECTION C – Prepared for publication, but not to date published</u>

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	
Stage of publication	Choose an item.

<u>SECTION D – Multi-authored work</u>

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I led the study design under the guidance of my supervisors D. Nitsch, B. Caplin and N. Pearce. I conducted the literature search, study selection, data extraction, quality assessment and data analysis. D. Nitsch screened a sample of 45 randomly selected titles and abstracts. I wrote the manuscript by following the advice provided by my supervisor. The manuscript was peer-reviewed, and I incorporated suggestions from reviewers into the final version

Student Signature: [redacted] **Date:** October 7/2018

Supervisor Signature: [redacted] **Date:** October 7/2018

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2.5 Research paper cover sheet: What do epidemiological studies tell us about chronic kidney disease of undetermined cause in Meso-America? A systematic review and meta-analysis

Clinical Kidney Journal

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Original Article

ORIGINAL ARTICLE

What do epidemiological studies tell us about chronic kidney disease of undetermined cause in Meso-America? A systematic review and meta-analysis

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Abstract

Background: The aim of this systematic review is to examine the epidemiological knowledge and gaps in understanding of the potential causes of chronic kidney disease of undetermined cause (CKDu) in Meso-America.

Methods: A systematic literature search of epidemiological studies of CKDu was conducted in PubMed, Embase and Web of Science from January 2000 to January 2017. Study quality was assessed by adapting the tool from Higgins *et al.* for observational studies. Where applicable, the summary prevalence odds ratio (POR) and 95% confidence interval (CI) were calculated using a random effects model.

Results: Twenty-five epidemiological studies were included in the analysis of risk factors for CKDu. The quality assessment of each occupational and community study was medium. The PORs for CKDu were males versus females 2.42 (95% CI 1.76–3.08), family history of CKD (versus none) 1.84 (95% CI 1.37–2.30), high water intake (versus low) 1.61 (95% CI 1.01–2.21) and low altitude (versus highland) 2.09 (95% CI 1.00–3.17). There were no significant associations between CKDu and pesticide exposure (versus no) 1.17 (95% CI 0.87–1.46), alcohol consumption (versus no) 1.34 (95% CI 0.84–1.84), non-steroidal anti-inflammatory drugs (versus no) 0.99 (95% CI 0.60–1.39) and heat stress (versus no) 1.52 (95% CI −0.91 – 3.95).

Conclusion: Our meta-analysis showed positive associations for males (versus females) and family history of CKD, water intake, lowland altitude and CKDu. There were no significant associations with pesticide exposure, non-steroidal anti-inflammatory drugs intake, heat stress and alcohol consumption.

Key words: CKDu, Meso-America, Meso-American nephropathy, meta-analysis, Nicaragua, risk factors, systematic review

Received: August 5, 2017. **Editorial decision:** October 18, 2017

Introduction

Meso-American nephropathy (MeN), also known as chronic kidney disease of undetermined aetiology (CKDu), is a growing public health problem and young agricultural workers of the Pacific coast of Meso-America have been the most affected group [1–3]. There was an increased recognition of this problem by researchers, universities and policymakers after 2000 due to a marked increase in mortality and morbidity [4].

Three years later, the Program on Work and Health in Central America (SALTRA) organized the first regional workshop on chronic kidney disease (CKD); this reviewed available data, including several studies that showed increased risks for CKD among sugarcane workers and high mortality related to CKD in particular areas in Nicaragua and El Salvador [4]. In 2014, the Pan American Health Organization classified CKDu as a major public health problem in Central America that requires urgent, effective and concerted multisectoral action [5].

In the last 10 years, several narrative reviews about CKDu have been published [6–9]. None of these have conducted a formal meta-analysis and there was no systematic assessment of the quality of the available evidence considering inherent limitations within the design and analyses of available epidemiological studies. The purpose of this study is therefore to formally examine the epidemiological knowledge and gaps in understanding of the potential causes of CKD of undetermined cause in Meso-America.

Materials and methods

Research strategy

We searched on PubMed, MEDLINE, Embase and Web Science to identify all original research that had been published between January 2005 and January 2017 reporting prevalence and mortality of CKDu in Meso-America. Search terms included a combination of text words and headings for 'Meso-American nephropathy', 'decreased kidney function', 'chronic kidney diseases of unknown cause', 'chronic kidney disease of non-traditional cause', 'agricultural', 'pesticide exposure', 'heat stress', etc., were used. The full search strategy is outlined in the Supplementary Material (Supplementary data, Tables S1–S3).

Inclusion and exclusion criteria

The search was limited to 'adolescent and adult human beings' and only papers published in English and Spanish languages were considered. The search was restricted to studies conducted in Meso-America (Central America and Mexico). The exposures of interest included heat stress, dehydration, pesticide, non-steroidal anti-inflammatory drugs (NSAIDs), workplace conditions, environmental toxins and infectious diseases. The outcomes of interest included the reduced estimated glomerular filtration rate (eGFR), elevated serum creatinine (SCr) and CKD of undetermined cause. A wide range of study designs were assessed, including cross-sectional studies, case–control studies, retrospective or prospective cohort studies and ecological studies. We excluded animal studies, editorials, systematic reviews and case reports (Supplementary data, Table S4). However, systematic reviews were used to manually search for references.

Selection process

All titles and abstracts were examined by one reviewer (M.G.-Q.) according to the above inclusion criteria. Any disagreement of some marginal cases were discussed between M.G.-Q., B.C. and D.N. After review of the titles and abstract were independently reviewed by two authors (M.G.-Q. and D.N.). All full-text articles were assessed independently using the same criteria and included if both reviewers recommended inclusion. A second reviewer (D.N.) checked a sample of 45 titles and abstracts selected randomly after duplicated articles were removed. Agreement between authors was quantified by j-statistic calculation.

Data extraction and quality assessment

A standardized data extraction form was used by M.G.-Q. to extract study characteristics: authors, study design, year, country, sample size, altitude, exposure and outcome definitions, main findings, strengths and limitations and confounding factors. Any difficulty in data extraction was discussed by joint review of the original papers.

Quality was assessed for each selected study using standard quality assessment tools for trials [10] that we adapted for observational studies. Studies were assigned a high, low or uncertain risk according to the following criteria: selection bias, non-differential measurement error for exposure and outcome, information bias in exposure and outcome, confounding and reverse causation.

Data synthesis and analysis

We reviewed the exposure and outcome definitions and reported risk factors in each study. Where there were several studies with similar exposure definitions, data were included in a random effects meta-analysis for the respective exposure and CKDu and displayed in a forest plot. Funnel plot analysis and Egger's test were performed to detect publication bias and $P < 0.05$ was considered significant.

The across-study heterogeneity was estimated by using Cochran's Q-statistic and calculating the proportion of total variability explained by heterogeneity (I^2) described by Higgins *et al.* [10]. All analyses were performed using Stata version 14 (StataCorp, College Station, TX, USA).

Results

The two reviewers had excellent agreement (Cohen' s $\kappa = 1$) on study inclusion after review of abstracts and titles. We identified 131 epidemiological studies on CKDu, of which 43% (56 papers) were duplicate studies using the same dataset. In addition, 53 studies did not meet the inclusion criteria and 3 were included by manual search, leaving 25 studies for the present systematic review (3 longitudinal occupational studies, 2 cross-sectional occupational studies, 14 community cross-sectional studies, 3 case–control studies and 3 ecological studies) (Fig. 1). The included studies were conducted from January 2000 to January 2017.

Occupational studies mainly assessed how occupational risk factors in the workplace were associated with an eGFR cross-sectionally or a subsequent decline of eGFR across harvest or a single cross-shift in younger sugarcane workers (Table 1). For many risk factors there is only one estimate per risk factor in each study (Supplementary data, Table S5).

We identified only two longitudinal community studies. One involved follow-up of eGFR measurements in the subgroup that previously had abnormal SCr results, thus incident disease was not captured [16]. For the other study, only baseline data are

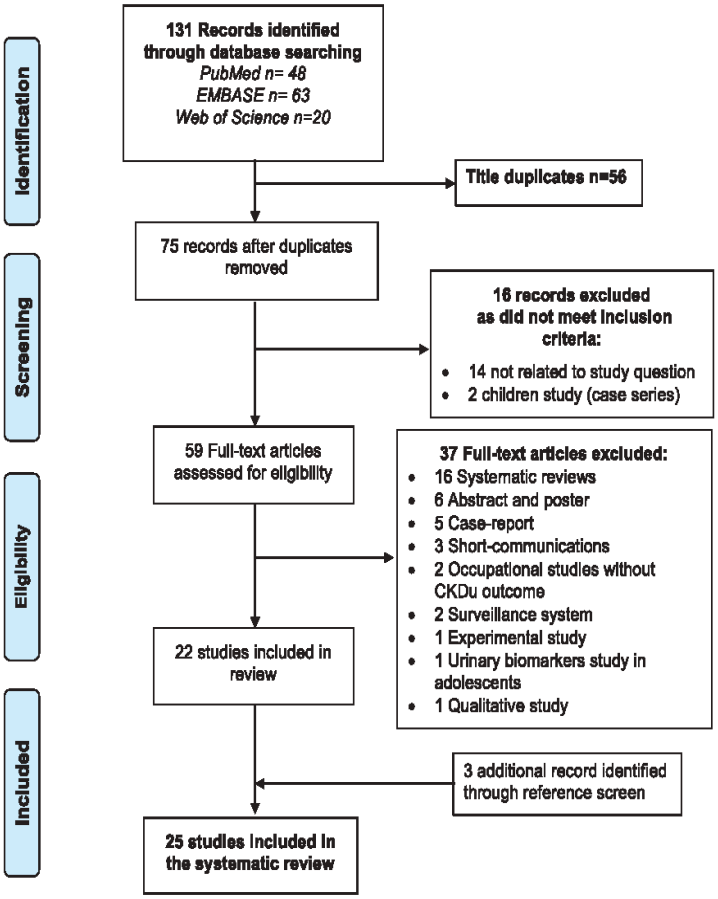

Fig. 1. CKD of undetermined cause (eGFR <60 mL/min/1.73 m^2), systematic review according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses diagram.

available [17]. Most of the community-based studies were of cross-sectional design and recruited participants from different age groups and explored a variety of exposures. Two-thirds of these used a similar outcome definition for kidney function involving calculating an eGFR (<60 mL/min/1.73 m^2) using the Modification of Diet in Renal Disease [18–25] or CKD Epidemiology Collaboration formula to quantify the prevalence of CKDu in the most affected regions [11–15, 26–28] (Table 2). Prevalence data among 14 cross-sectional community studies are confounded by age, as people with different ages were included, but overall more men than women were affected (Fig. 2). It was not possible to report an age-standardized CKDu prevalence because a breakdown of the findings by age was not available for most studies.

The quality of three longitudinal occupational studies was affected by severe loss of follow-up (up to 50%) during the study period, either due to changes in role or redundancy [11–13] (Table 3 and rationale in Supplementary data, Table S6). One cross-sectional occupational study [15] and cross-sectional community-based studies [23–25, 31, 32] had incomplete adjustment for confounding and reverse causation. One case–control study suffered from selection bias of participants, because researchers used improper procedures for selecting their cases and controls (volunteer participations at clinics) (Table 4). Many cross-sectional studies were limited to single measurements of creatinine, thus not fulfilling the chronicity criterion for CKD. Furthermore, the quality of three ecological studies was potentially affected by unmeasured confounding factors and within-regions variability in exposure and disease classification (outcome) (Table 4 and rationale in Supplementary data, Table S7).

Sufficient data for meta-analysis were available for a subset of risk factors: male sex, family history of CKD, water intake, pesticide exposure, alcohol consumption, self-medication with NSAIDs, heat stress and altitude. These were from 10 cross-sectional community studies and 3 case–control community studies. In the meta-analysis, eight cross-sectional community studies showed positive associations between male and eGFR <60 mL/min/1.73 m^2 [prevalence odds ratio [POR] 2.42 [95% confidence interval (CI) 1.76–3.08]; Cochran's Q-statistic $P = 0.056$, $I^2 = 49.0\%$]. Three studies showed strong associations between family history of kidney disease and eGFR <60 mL/min/1.73 m^2 [POR 1.84 (95% CI 1.37–2.30); Cochran's Q-statistic $P = 0.947$, $I^2 = 0.0\%$]. For high water intake and eGFR <60 mL/min/1.73 m^2 the POR was 1.61 (95% CI: 1.01–2.21; Cochran's Q-statistic $P = 0.511$, $I^2 = 0.0\%$) and for lowland altitude (versus highland) it was 2.09 (95% CI 1.00–3.17; Cochran's Q-statistic $P = 0.272$, $I^2 = 23.3\%$). The pooled POR for pesticide exposure was 1.17 (95% CI 0.87–1.46; Cochran's Q-statistic $P = 0.537$, $I^2 = 0.0\%$) (Fig. 3). The summary estimate for alcohol consumption (yes versus no) was 1.34 (95% CI 0.84–1.84; Cochran's Q-statistic $P = 0.088$, $I^2 = 45.5\%$), for NSAIDs intake (yes versus no) and eGFR <60 mL/min it was close to 1 [POR 0.99 (95% CI 0.60–1.39); Cochran's Q-statistic $P = 0.399$, $I^2 = 0.0\%$] and for heat stress exposure (yes versus no) it was 1.52 (95% CI −0.91 to 3.95; Cochran's Q-statistic $P = 0.065$, $I^2 = 70.6\%$) (Supplementary data,

Table 1. Characteristics of occupational studies included in the systematic review (n=5)

					Sample size/sex					
Authors	Year	Design	Country	Region	*N*	Male	Female	Age (years)	Exposures	Outcome definition
Wesseling *et al.* [11]	2016	Cohort	Nicaragua	León and Chinandega	54	54	–	17–38	Sugarcane cutters, anthropometrics measurements, fructose intake and urinary biomarkers	Decline in eGFR over time
Laws *et al.*[a] [12]	2016	Cohort	Nicaragua	Chinandega	284	251	33	18–63	Job category of sugarcane harvesters, years worked at sugar mill, water intake, isotonic solution intake, age and sex	Kidney urinary biomarkers and kidney function over harvest
Laws *et al.*[a] [13]	2015	Cohort	Nicaragua	Chinandega	284	251	33	18–63	Job category of sugarcane harvesters, years worked at sugar mill, water intake, isotonic solution intake, age and sex	Decline in eGFR over harvest
Wesseling *et al.* [14]	2016	Cross-sectional	Nicaragua	León and Chinandega	194	194	–	17–39	Differences between three occupations, socioeconomic status, hydration, lifestyle and health risk factors	eGFR <80 mL/min/1.73 m^2
Garcia-Trabanino *et al.* [15]	2015	Cross-sectional	El Salvador	Suchitoto, El Paisnal and San Luis Talpa	189	168	21	18–49	Sugarcane workers, workplace conditions, dehydration, heat stress, pesticide exposure and anthropometric measurements	Cross-shift changes in eGFR

[a]The baseline sample was 1104 sugarcane workers, but at the end of the harvest it was 284.
eGFR, estimated glomerular filtration rate.

Table 2. Characteristics of community-based studies included in the systematic review (n=20)

Authors	Year	Design	Country	Region	Sample size/sex			Age (years)	Exposures	Outcome definition
					N	Male	Female			
Nicaragua										
González-Quiroz *et al.*[a] [17]	2017	Cohort	Nicaragua	León and Chinandega	350	263	87	18–30	Sociodemographic information, work history, lifestyle, work conditions, liquid intake and current diseases (hypertension or diabetes)	eGFR <90 mL/min/1.73 m^2
Minnings *et al.* [16]	2016	Cohort	Nicaragua	Rivas	1242	537	705	≥18	Demographic data, household membership, health symptoms, hydration practices, occupational and exposure history and personal medical history	SCr >1.5 mg/dL if male and >1.2 mg/dL if female or eGFR <60 mL/min/1.73 m^2
Kupferman *et al.* [27]	2016	Cross-sectional	Nicaragua	Chichigalpa	226	178	88	≥18	Clinical and demographic characteristics	SCr >1.5 mg/dL if male and >1.2 mg/dL if female or eGFR <60 mL/min/1.73 m^2
Lebov *et al.* [29]	2015	Cross-sectional	Nicaragua	León	2275	1324	951	18–70	Demographic indicators, source of drinking water, personal medical history, occupation and lifestyle	eGFR <60 mL/min/1.73 m^2
Laux *et al.*[b] [21]	2012	Cross-sectional	Nicaragua	Matagalpa	267	120	147	20–60	Demographic data, personal and family medical history, medications, occupation and lifestyle	eGFR <60 mL/min/1.73 m^2
Torres *et al.*[c] [18]	2010	Cross-sectional	Nicaragua	León and Chinandega	1096	479	617	20–60	Sociodemographic data, personal medical history (diabetes, hypertension, obesity and renal lithiasis), NSAIDs and occupation	SCr >1.2 mg/dL if male and >0.9 mg/dL if female or eGFR <60 mL/min/1.73 m^2
González-Quiroz[b] [30]	2010	Cross-sectional	Nicaragua	Chichigalpa	704	237	467	20–60	Sociodemographic data, personal medical history (diabetes, hypertension, obesity and renal lithiasis), alcohol, NSAIDs, occupation and pesticide exposure	eGFR <60 mL/min/1.73 m^2
Raines *et al.*[a] [26]	2014	Case–control	Nicaragua	Chichigalpa	424	166	258	15–69	Demographic data, personal medical history, lifestyle, NSAIDs, cane chewing, inhaled pesticides, water intake, sugar beverage intake, occupation and personal protective equipment	eGFR <60 mL/min/1.73 m^2
O'Donnell *et al.*[a] [20]	2010	Case–control	Nicaragua	Quezalguaque	771	298	473	≥18	Age, sex, anthropometric measurements, education level, work history, exposure to pesticides, alcohol, cigarrete use and family and personal medical history	eGFR <60 mL/min/1.73 m^2
Sanoff *et al.*[a] [19]	2010	Case–control	Nicaragua	Chinandega	997	848	149	≥18	Demographic data, hypertension, diabetes, family history of kidney disease and occupational and non-occupational exposures	eGFR <60 mL/min/1.73 m^2
El Salvador										
Orantes-Navarro *et al.*[d] [24]	2016	Cross-sectional	El Salvador	Bajo Lempa, Guayapa, Las Brisas	2115	1058	1057	<18	Age, sex and region	eGFR <60 mL/min/1.73 m^2, by a second measurement of SCr within 3 months' difference

(continued)

Table 2. Continued

Authors	Year	Design	Country	Region	Sample size/sex			Age (years)	Exposures	Outcome definition
					N	Male	Female			
Orantes-Navarro *et al.*[d] [31]	2015	Cross-sectional	El Salvador	Bajo Lempa, Guayapa, Las Brisas	1412	–	1412	≥18	Age, sex, clinical history (hypertension and diabetes), family history (CKD, diabetes and hypertension), occupation, agrochemicals exposure and physical examination (weight, height and blood pressure)	eGFR <60 mL/min/1.73 m^2, by a second measurement of SCr within 3 months' difference
Vela *et al.*[d] [25]	2014	Cross-sectional	El Salvador	El Jicaro and Dimas Gutiérrez	223	110	113	≥15	Age, sex, physical measurements (weight, height, abdominal circumference and blood pressure), personal and family medical history, occupational and environmental exposures	eGFR <60 mL/min/1.73 m^2, by a second measurement of SCr within 3 months' difference
Orantes *et al.*[a,d] [23]	2014	Cross-sectional	El Salvador	Bajo Lempa, Guayapa abajo, Las Brisas	2388	976	1412	≥18	Age, sex, physical measurements (weight, height, waist circumference and blood pressure), personal and family medical history, occupational exposures and lifestyle	eGFR <60 mL/min/1.73 m^2, by a second measurement of SCr within 3 months' difference
Peraza *et al.*[b] [28]	2012	Cross-sectional	El Salvador	San Luis Talpa, Jiquilisco, Apastepeque, San Salvador and Ataco	664	256	408	20–60	Demographic data, occupational exposure, medical conditions and lifestyle	SCr >1.2 mg/dL if male and >0.9 mg/dL if female or eGFR <60 mL/min/1.73 m^2
Orantes *et al.*[a,d] [22]	2011	Cross-sectional	El Salvador	Bajo Lempa	775	343	432	≥18	Age, sex, physical measurements (weight, height, abdominal circumference and blood pressure), personal and family medical history, occupational, environmental exposures and lifestyle	eGFR <60 mL/min/1.73 m^2, by a second measurement of SCr within 3 months' difference
Garcia-Trabanino *et al.*[a] [32]	2005	Cross-sectional	El Salvador	Jaquilisco	291	291	–	34–66	Age, place of residence, occupation in agricultural work, history of pesticides exposure, alcohol consumption and basic medical history	SCr >1.5 mg/dL
Costa Rica, El Salvador and Guatemala										
Wesseling *et al.* [33]	2015	Ecological	Costa Rica	Costa Rica	6295	3843	2452	≥20	Age, sex, region, altitude, climate and sugarcane production	CKD by death certificate
Laux *et al.* [34]	2015	Ecological	Guatemala	Guatemala	3105	1591	1514	Not reported	Sex, sugarcane cultivation, temperature, region, life expectancy, educational level and wealth	CKD by medical records from an RRT programme
VanDervort *et al.* [35]	2014	Ecological	El Salvador	El Salvador	24 726	–	–	No reported	Temperature, crops (sugarcane, sorghum, corn, beans, cotton and coffee)	CKD by medical records from National Surveillance Health System

[a]Altitude: at sea level.
[b]More than 500 m above sea level.
[c]At sea level and >500 m above sea level.
[d]Five studies from El Salvador applied the CKD definition: persistence of renal damage markers for ≥3 months or GFR <60 mL/min/1.73 m^2.
eGFR, estimated glomerular filtration rate; SCr, serum creatinine; CKD, chronic kidney disease; NSAIDs, non-steroidal antiinflammatory drugs; RRT, renal replacement therapy.

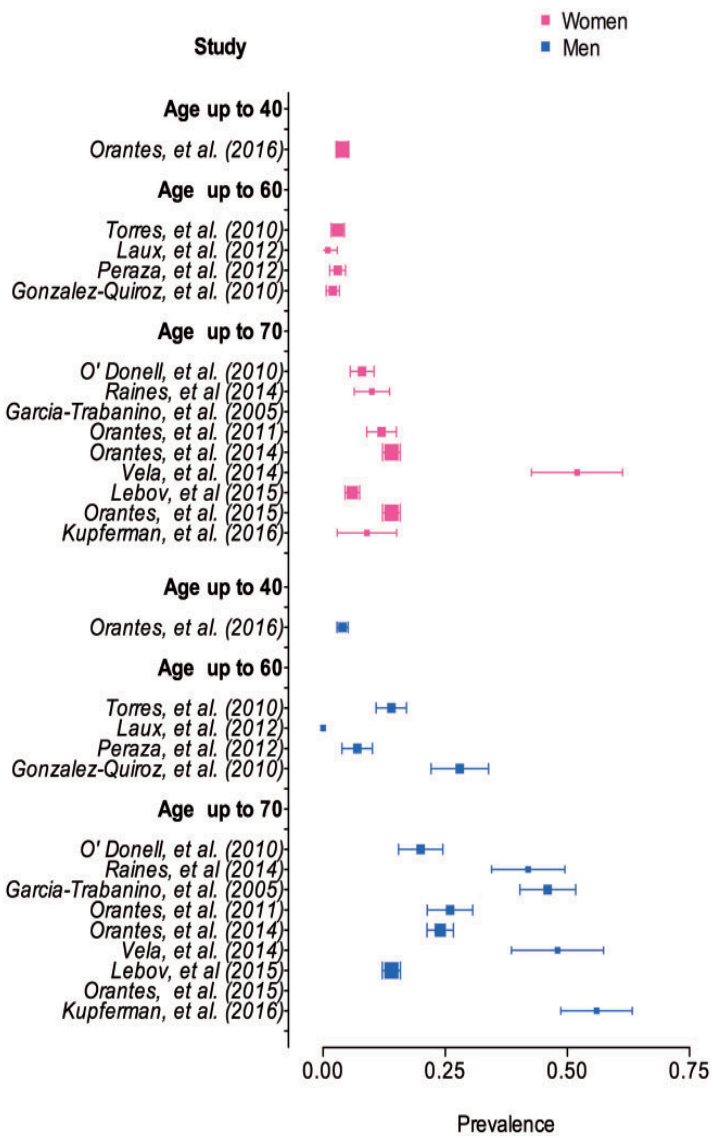

Fig. 2. Forest plot of all prevalence of chronic kidney disease of undetermined cause (eGFR <60 mL/min/1.73 m^2) by age group and sex from 14 cross-sectional community studies identified.

Fig. S1). A forest plot of occupation was not included because each study used different reference categories.

We tested for publication bias for sex, pesticide exposure and alcohol consumption risk factors. The funnel plot for studies that have assessed the above risk factors provides evidence for potential publication bias for pesticide exposure and alcohol consumption ($P < 0.014$ and $P < 0.048$, respectively) (Supplementary data, Fig. 2).

Discussion

We found 25 epidemiological studies that estimated the prevalence and assessed risk factors for CKDu in Meso-America. Our meta-analysis found a clear positive association between male sex, family history of CKD, high water intake, lowland altitude and reduced eGFR <60 mL/min/1.73 m^2. There was no evidence for associations with pesticide exposure, NSAIDs intake, alcohol consumption and heat stress. The quality of cross-sectional studies was medium due to the potential for reverse causality, incomplete adjustment for confounding factors and the use of a single SCr measurement. Longitudinal occupational studies were affected by severe loss of follow-up.

A major issue impacting the quality of all the studies examined is that CKDu prevalence was estimated using a single SCr measurement rather than two measurements at least 3 months apart [36]. In affluent countries, a single measurement is frequently used to estimate the prevalence of CKD, as the intra-individual variability of creatinine under stable conditions is only a few percent. However, in a hot setting there is considerable seasonal variation and variation depending on work patterns and dehydration status; therefore, depending on when people are measured, they may have short-term fluctuations of creatinine that are far more pronounced than in cooler settings. Also, creatinine elevation can occur due to variations in factors such as exercise, muscle mass and diet. These factors may not only affect variability within individuals, but may also bias comparisons across populations studies, since each study may over- or underestimate kidney function depending on the season and

Table 3. Quality assessment of occupational studies (n–5)[a]

Studies	Selection bias: participation	Selection bias: loss of follow-up	Non-differential misclassification exposure	Information bias of exposure	Non-differential misclassification of outcome	Information bias of outcome	Confounding	Reverse causation
Cohort studies								
Wesseling *et al.* [11]								
Laws *et al.* [12]								
Laws *et al.* [13]								
Cross-sectional studies								
Wesseling *et al.* [14]		N/A						
Garcia-Trabanino *et al.* [15]		N/A						

[a]Green bars: low risk of bias; yellow bars: medium risk of bias; red bars: high risk of bias.
N/A, not applicable.

Table 4. Quality assessment of community-based studies (n–20)[a]

Studies	Selection bias: participation	Selection bias: loss of follow-up	Non-differential misclassification exposure	Information bias of exposure	Non-differential misclassification of outcome	Information bias of outcome	Confounding	Reverse causation
Cohort studies								
González-Quiroz *et al.* [17]		N/A						
Minnings *et al.* [16]								
Cross-sectional studies								
Orantes-Navarro *et al.* [24]		N/A	N/R	N/R				N/R
Kupferman *et al.* [27]		N/A						
Orantes-Navarro *et al.* [31]		N/A						
Lebov *et al.* [29]		N/A						
Vela *et al.* [25]		N/A						
Orantes *et al.* [23]		N/A						
Peraza *et al.* [28]		N/A						
Laux *et al.* [21]		N/A						
Orantes *et al.* [22]		N/A						
Torres *et al.* [18]		N/A						
Gonzalez-Quiroz [30]		N/A						
Garcia-Trabanino *et al.* [32]		N/A						
Case–control studies								
Raines *et al.* [26]		N/A						
O'Donnell *et al.* [20]		N/A						
Sanoff *et al.* [19]		N/A						
Ecological studies								
Wesseling *et al.* [33]		N/A						
Laux *et al.* [34]		N/A						
VanDervort *et al.* [35]		N/A						

[a]Green bars: low risk of bias; yellow bars: medium risk of bias; red bars: high risk of bias.
N/A, not applicable; N/R, not reported.

setting of fieldwork or biological variation in the production of creatinine. In addition, creatinine levels may also be affected by 'fixed' factors such as ethnicity, which may also bias comparisons between populations.

The longitudinal occupational studies were affected by a loss of follow-up of up to 50% of their participants. This severely compromises study validity because those with CKDu are more likely to not be followed up [11–13]. Occupational studies are used to increase the power of a study when it is thought that a particular occupational exposure causes a problem. However, in the context of CKDu, it is not yet entirely clear whether occupation is the only risk factor or whether there are other risk factors that predispose young men to CKDu when they start working in sugarcane. Overall, considering the differential loss to follow-up of occupational studies, community cohorts have many advantages compared with occupational studies since they represent the entire risk population (workers from all occupations and both genders) and an assessment of environmental exposures at home [17].

Ecological studies may be affected by variability within regions in exposure and disease classification and by unmeasured confounding factors [33–35]. CKD mortality rate may vary across regions because of misclassification either of the cause reported by death certificate or by better case detection.

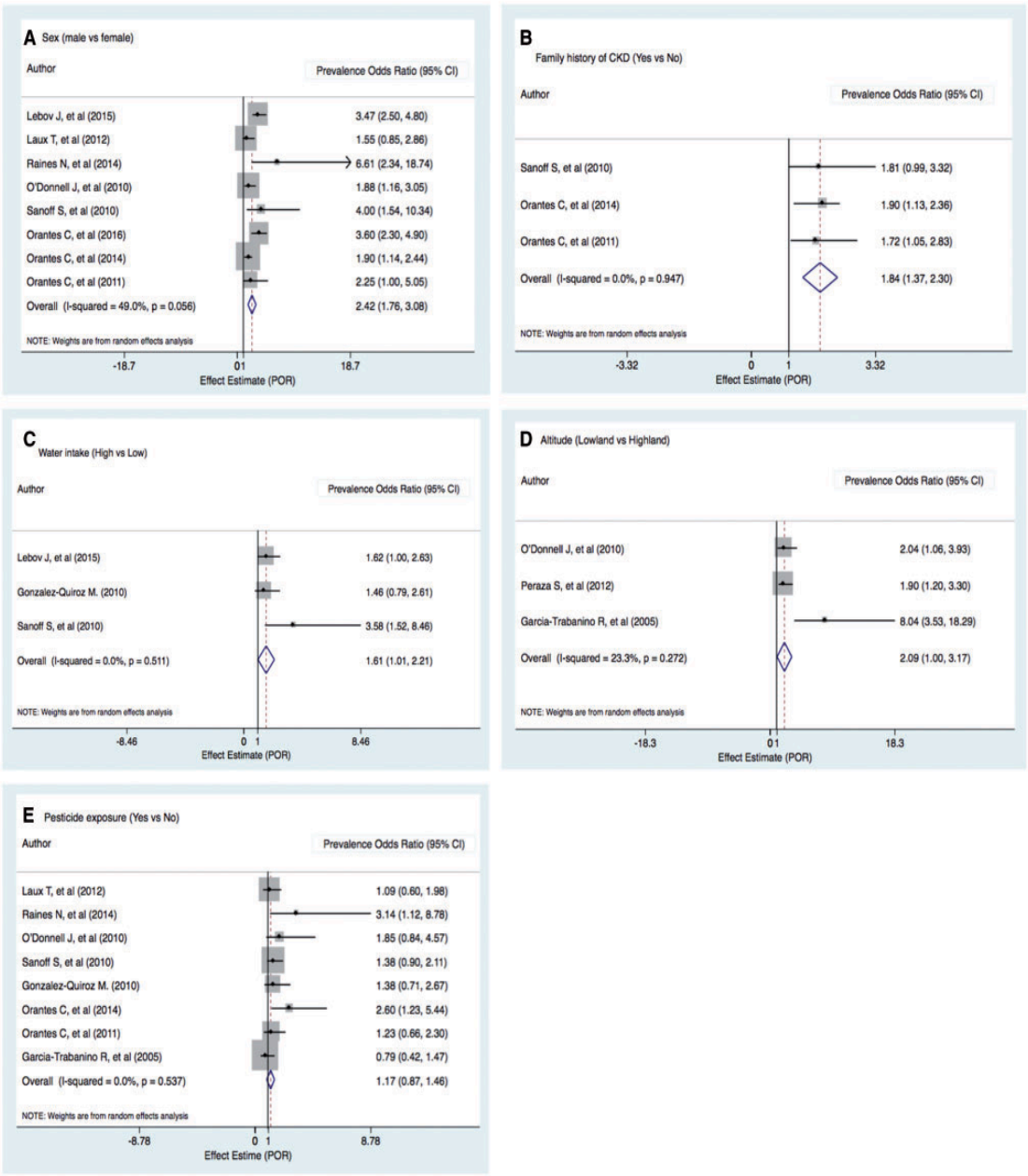

Fig. 3. Forest plots of association with (A) sex, (B) family history of CKD, (C) water intake, (D) lowland altitude and (E) pesticide exposure estimates associated with CKD of undetermined caused (eGFR <60 mL/min/1.73 m^2). Black diamond data markers express PORs; horizontal lines are the 95% CIs; grey square marker size indicating the statistical weight of the study using the random effects meta-analysis. A diamond data marker denotes the overall POR and 95% for the outcome of interest.

Moreover, environmental temperature may be different within regions or areas due to variability in seasons and altitude. Finally, the lack of control for confounders have been an Achilles heel for ecological studies, even on the assumption that all variables have been accurately measured for all groups at a national level, basically due to the analysis strategy, which cannot completely remove bias due to the confounder.

Epidemiological studies have underlined many potential risk factors for CKDu, including male sex, occupation, high ambient temperature, self-medication with NSAIDs, altitude, exposure to heavy metals or pesticides and genetic susceptibility [11, 13–15, 18, 28]. While our systematic review could confirm the association with male sex, none of the other suggested risk factors were sufficiently well studied to conclusively prove or disprove their role. The most commonly cited working hypothesis for this disease has been heat stress causing repetitive episodes of dehydration in agricultural and non-agricultural workers due to working under heat stress and high humidity [15, 37], which may result in acute kidney injury (AKI) secondary to hypoperfusion or rhabdomyolysis [38]. However, although this hypothesis has been explored in an experimental study that suggested that dehydration and hyperosmolarity may induce tubular injury via activation of the polyolfructokinase pathway in the kidney [39], there have been no corresponding data in humans to support this hypothesis.

Our meta-analysis has identified positive associations of high water intake and CKDu in two cross-sectional community studies [29, 30] and one case–control study [19]. The study authors' interpretation of these findings was that high water intake could be a proxy for exposure to heat stress and volume depletion during the workday secondary to high exertion and sweating [11, 14, 15, 40]. Some authors hypothesized that high water intake means that study participants drank more water trying to compensate for fluid deprivation, but that this is not enough to recover their hydration status [11, 14, 15]. Other authors have suggested that these associations are driven by intake from contaminated water sources (with pesticides or heavy metals) in the affected areas [41, 42]. An alternative interpretation could be reverse causation due to underlying kidney damage, in that those with kidney damage are unable to concentrate their urine and therefore need to drink more to not feel thirsty. To address the issue, it will be important to conduct more longitudinal studies to gain better insight into this association.

Pesticides are used extensively in Meso-America. Farmers in the cooler highland regions use pesticides similarly to farmers in coastal regions, yet CKDu prevalence is much lower at higher altitudes [15, 20–23, 26, 30]. Most of the studies that suggest a possible association between AKI and exposure to organochlorides, paraquat, 2,4-diclorophenoxyacetic and glyphosate have been conducted in animals [43, 44]. A single prospective cohort study among male licensed pesticide applicators in the USA reported an association between end-stage renal disease and exposure–response and increasing accumulated lifetime days in pesticide exposure and non-exposure for some herbicides such as alachlor, paraquat, pendimethalin, atrazine, permethrin and metolochlor [45]. The principal limitation of existing epidemiological studies is that exposure has been assessed using categorical questions (yes and no) and not by quantifying the pesticide residues in urine or blood [14, 19, 20, 26, 32]. Our findings suggest selective reporting of studies supporting an association with CKDu. Overall, the evidence about pesticide exposure and CKDu is still inconclusive.

Genetic predisposition may play a role in the CKDu epidemic, as some studies, and our meta-analysis, have suggested a positive association between family history of CKD and CKDu. Although CKD in general shows a high heritability of disease, suggesting familial clustering of risk factors, these have not been explained by genetic association studies [46, 47]. A positive association with family history of CKDu may simply be due to children who lost parents to CKDu or living in rural areas starting to work earlier in sugarcane or agriculture to support their household income.

Our systematic review has strengths and limitations. To our knowledge, this is the first systematic review that included a meta-analysis and evaluated the study quality of each epidemiological study by using a pre-specified tool adapted from Higgins *et al.* [10] for observational studies. Second, we included a broad definition of CKD of unknown aetiology and a variety of exposures. The main limitations of the review are that the available evidence on CKDu is overall patchy and inconclusive.

In summary, apart from male sex, positive family history, high water intake and lowland altitude, existing studies have been inconclusive with regards to potential risk factors for CKDu, such as pesticide use, NSAIDs, heavy metals, alcohol consumption, heat stress and dehydration. Longitudinal community-based studies are needed to address problems of reverse causality (as per existing cross-sectional studies) as well as differential loss to follow-up (as per existing occupational studies).

Authors' contributions

This study was conceived and designed by M.G.-Q., N.P., B.C. and D.N. Data collection was performed by M.G.-Q. and B.C. The analysis and interpretation of the results were done by M.G.-Q., B.C. and D.N. The draft was written by M.G.-Q. and D.N. All authors read and approved the final manuscript.

Supplementary data

Supplementary data are available at ckj online.

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Conflict of interest statement

None declared.

References

1. Weiner DE, McClean MD, Kaufman JS *et al.* The Central American epidemic of CKD. *Clin J Am Soc Nephrol* 2013; 8: 504–511
2. Correa-Rotter R, Wesseling C, Johnson RJ. CKD of unknown origin in Central America: the case for a Mesoamerican nephropathy. *Am J Kidney Dis* 2014; 63: 506–520
3. Wegman D, Crowe J, Hogstedt C *et al.* (eds). *Mesoamerican nephropathy: report from the second international research workshop on MeN. ISBN 978-9968-924-33-7.* Heredia, Costa Rica: SALTRA/IRET-UNA, 2016
4. Cuadra SN, Kristina J, Christer H *et al Chronic Kidney Disease: Assessment of Current Knowledge and Feasibility for Regional Research Collaboration in Central America.* Heredia, Costa Rica: SALTRA, 2006
5. Pan American Health Organization–World Health Organization. *Resolution CD52.R1: Chronic Kidney Disease in Agricultural Communities in Central America.* Washigton, DC: PAHO, 2014. http://www.paho.org/hq/index.php? option=com_content&view=article&id=8833&Itemid=40033&lang=en (last accessed 10 June 2017)
6. Lunyera J, Mohottige D, Isenburg MV *et al.* CKD of uncertain etiology: a systematic review. *Clin J Am Soc Nephrol* 2016; 11: 379–385
7. Gifford FJ, Gifford RM, Eddleston M *et al.* Endemic nephropathy around the world. *Kidney Int Rep* 2017; 2: 282–292
8. Madero M, García-Arroyo FE, Sánchez-Lozada LG. Pathophysiologic insight into MesoAmerican nephropathy. *Curr Opin Nephrol Hypertens* 2017; 26: 296–302
9. Elinder C-G, Wijkström A, Wijkstrom J. Mesoamerican nephropathy (MeN): a 'new' chronic kidney disease related to occupational heat exposure with repeated deprivation of salts and water. *Int J Nephrol Kidney Failure* 2015; 1: 1–9
10. Higgins JP, Altman DG, Gøtzsche PC *et al.* The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 2011; 343: d5928
11. Wesseling C, Aragón A, González M *et al.* Kidney function in sugarcane cutters in Nicaragua—a longitudinal study of

workers at risk of Mesoamerican nephropathy. *Environ Res* 2016; 147: 125–132
12. Laws RL, Brooks DR, Amador JJ *et al.* Biomarkers of kidney injury among nicaraguan sugarcane workers. *Am J Kidney Dis* 2016; 67: 209–217
13. Laws RL, Brooks DR, Amador JJ *et al.* Changes in kidney function among Nicaraguan sugarcane workers. *Int J Occup Environ Health* 2015; 21: 241–250
14. Wesseling C, Aragón A, González M *et al.* Heat stress, hydration and uric acid: a cross-sectional study in workers of three occupations in a hotspot of Mesoamerican nephropathy in Nicaragua. *BMJ Open* 2016; 6: 1–12
15. García-Trabanino R, Jarquín E, Wesseling C *et al.* Heat stress, dehydration, and kidney function in sugarcane cutters in El Salvador—a cross-shift study of workers at risk of Mesoamerican nephropathy. *Environ Res* 2015; 142: 746–755
16. Minnings K, Fiore M, Mosco M *et al.* The Rivas Cohort Study: design and baseline characteristics of a Nicaraguan cohort. *BMC Nephrol* 2016; 17: 93
17. González-Quiroz M, Camacho A, Faber D *et al.* Rationale, description and baseline findings of a community-based prospective cohort study of kidney function amongst the young rural population of Northwest Nicaragua. *BMC Nephrol* 2017; 18: 16
18. Torres C, Aragón A, González M *et al.* Decreased kidney function of unknown cause in Nicaragua: a community-based survey. *Am J Kidney Dis* 2010; 55: 485–496
19. Sanoff SL, Callejas L, Alonso CD *et al.* Positive association of renal insufficiency with agriculture employment and unregulated alcohol consumption in Nicaragua. *Ren Fail* 2010; 32: 766–777
20. O'Donnell JK, Tobey M, Weiner DE *et al.* Prevalence of and risk factors for chronic kidney disease in rural Nicaragua. *Nephrol Dial Transplant* 2011; 26: 2798–2805
21. Laux TS, Bert PJ, Barreto Ruiz GM *et al.* Nicaragua revisited: evidence of lower prevalence of chronic kidney disease in a high-altitude, coffee-growing village. *J Nephrol* 2012; 25: 533–540
22. Orantes CM, Herrera R, Almaguer M *et al.* Chronic kidney disease and associated risk factors in the Bajo Lempa region of El Salvador: Nefrolempa study, 2009. *MEDICC Rev* 2011; 13: 14–22
23. Orantes CM, Herrera R, Almaguer M *et al.* Epidemiology of chronic kidney disease in adults of Salvadoran agricultural communities. *MEDICC Rev* 2014; 16: 23–30
24. Orantes-Navarro CM, Herrera-Valdes R, Almaguer-Lopez M *et al.* Chronic kidney disease in children and adolescents in Salvadoran farming communities: NefroSalva Pediatric Study (2009–2011). *MEDICC Rev* 2016; 18; 15
25. Vela XF, Henriquez DO, Zelaya SM *et al.* Chronic kidney disease and associated risk factors in two Salvadoran farming communities, 2012. *MEDICC Rev* 2014; 16: 55–60
26. Raines N, Gonzalez M, Wyatt C *et al.* Risk factors for reduced glomerular filtration rate in a Nicaraguan community affected by Mesoamerican nephropathy. *MEDICC Rev* 2014; 16: 16–22
27. Kupferman J, Amador JJ, Lynch KE *et al.* Characterization of Mesoamerican nephropathy in a kidney failure hotspot in Nicaragua. *Am J Kidney Dis* 2016; 68: 716–725
28. Peraza S, Wesseling C, Aragon A *et al.* Decreased kidney function among agricultural workers in El Salvador. *Am J Kidney Dis* 2012; 59: 531–540
29. Lebov JF, Valladares E, Peña R *et al.* A population-based study of prevalence and risk factors of chronic kidney disease in Leon, Nicaragua. *Can J Kidney Health Dis* 2015; 2: 6
30. Gonzalez-Quiroz M. Enfermedad Renal Crónica: prevalencia y factores de riesgo ocupacionales en el municipio de Chichigalpa [Tesis de postgrado]. Universidad Nacional Autónoma de Nicaragua, León, 2010
31. Orantes Navarro CM, Herrera Valdés R, López MA *et al.* Epidemiological characteristics of chronic kidney disease of non-traditional causes in women of agricultural communities of El Salvador. *Clin Nephrol* 2015; 83(Suppl 1): 24–31
32. Gracia-Trabanino R, Dominguez J, Jansa JM *et al.* [Proteinuria and chronic renal failure in the coast of El Salvador: detection with low cost methods and associated factors]. *Nefrologia* 2005; 25: 31–38
33. Wesseling C, van Wendel de Joode B, Crowe J *et al.* Mesoamerican nephropathy: geographical distribution and time trends of chronic kidney disease mortality between 1970 and 2012 in Costa Rica. *Occup Environ Med* 2015; 72: 714–721
34. Laux TS, Barnoya J, Guerrero DR *et al.* Dialysis enrollment patterns in Guatemala: evidence of the chronic kidney disease of non-traditional causes epidemic in Mesoamerica. *BMC Nephrol* 2015; 16: 54
35. VanDervort DR, Lopez DL, Orantes CM *et al* Spatial distribution of unspecified chronic kidney disease in El Salvador by crop area cultivated and ambient temperature. *MEDICC Rev* 2014; 16: 31–38
36. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int Suppl* 2013; 3: 1–150
37. Crowe J, Wesseling C, Solano BR *et al.* Heat exposure in sugarcane harvesters in Costa Rica. *Am J Ind Med* 2013; 56: 1157–1164
38. Paula Santos U, Zanetta DM, Terra-Filho M *et al* Burnt sugarcane harvesting is associated with acute renal dysfunction. *Kidney Int* 2015; 87: 792–799
39. Roncal Jimenez CA, Ishimoto T, Lanaspa MA *et al.* Fructokinase activity mediates dehydration-induced renal injury. *Kidney Int* 2014; 86: 294–302
40. Lucas RA, Bodin T, García-Trabanino R *et al.* Heat stress and workload associated with sugarcane cutting—an excessively strenuous occupation! *Extrem Physiol Med* 2015; 4(Suppl 1): A23
41. Jayasumana C, Gunatilake S, Senanayake P. Glyphosate, hard water and nephrotoxic metals: are they the culprits behind the epidemic of chronic kidney disease of unknown etiology in Sri Lanka? *Int J Environ Res Public Health* 2014; 11: 2125–2147
42. Jayasumana C, Paranagama P, Agampodi S *et al.* Drinking well water and occupational exposure to Herbicides is associated with chronic kidney disease, in Padavi-Sripura, Sri Lanka. *Environ Health* 2015; 14: 6
43. Uyanikgil Y, Ateş U, Baka M *et al.* Immunohistochemical and histopathological evaluation of 2,4-dichlorophenoxyacetic acid-induced changes in rat kidney cortex. *Bull Environ Contam Toxicol* 2009; 82: 749–755
44. Poovala VS, Huang H, Salahudeen AK. Role of reactive oxygen metabolites in organophosphate–bidrin-induced renal tubular cytotoxicity. *J Am Soc Nephrol* 1999; 10: 1746–1752
45. Lebov JF, Engel LS, Richardson D *et al.* Pesticide use and risk of end-stage renal disease among licensed pesticide applicators in the Agricultural Health Study. *Occup Environ Med* 2016; 73: 3–12
46. Wuttke M, Kottgen A. Insights into kidney diseases from genome-wide association studies. *Nat Rev Nephrol* 2016; 12: 549–562
47. Gorski M, van der Most PJ, Teumer A *et al.* 1000 Genomes-based meta-analysis identifies 10 novel loci for kidney function. *Sci Rep* 2017; 7: 45040

2.6 Supplementary material

Supplementary table 1: PubMed search strategy.

Search	Results	Search type
1	("Kidney Diseases"[Mesh] OR "Chronic Kidney Disease"[tiab] OR "chronic kidney disease"[tiab] OR "chronic kidney diseases"[tiab] OR "chronic renal disease"[tiab] OR "chronic renal diseases"[tiab] OR ckdu[tiab])	462868
2	("Mesoamerican Nephropathy"[Mesh] OR "mesoamerican nephropathy"[tiab] OR "Decreased Kidney Function"[tiab] OR "decreased kidney function"[tiab] OR "Chronic Kidney Disease"[tiab] OR "Chronic Kidney Disease of non-traditional"[tiab] OR "chronic kidney disease of unknown"[tiab] OR "Nefrolempa"[tiab] OR ckdu[tiab] OR ckdnt[tiab])	30799
3	("Agriculture"[Mesh] OR "Chemistry, Agricultural"[Mesh] OR "Agricultural Workers' Diseases"[Mesh] OR "Pesticides"[Mesh] OR "Pesticides" [Pharmacological Action] OR "Pesticide Residues"[Mesh] OR "Agrochemicals"[Mesh] OR "Metals, Heavy"[Mesh:NoExp] OR "Aluminum"[Mesh] OR "Fluorides"[Mesh] OR "Arsenic"[Mesh] OR "Cadmium"[Mesh] OR "Lead"[Mesh] OR "Groundwater"[Mesh] OR “agriculture"[tiab] OR “agricultural"[tiab] OR “farm"[tiab] OR “pesticide"[tiab] OR “pesticides"[tiab] OR “agrochemical"[tiab] OR “heavy metal”[tiab] OR “heavy metals”[tiab] OR "Aluminum"[tiab] OR "Fluorides"[tiab] OR "fluoride"[tiab] OR "Arsenic"[tiab] OR "Cadmium"[tiab] OR “dehydration”[tiab] OR “heat stress”[tiab] OR “Heat Stress”[tiab] OR “Heat-Related”[tiab] OR “heat related”[tiab] OR “Heat exposure”[tiab] OR “Sugarcane”[tiab] OR “sugarcane”[tiab] OR “harvesters”[tiab] OR “NSAIDs”[tiab] OR "Groundwater"[tiab] OR “ground water”[tiab] OR “hard water”[tiab] OR (water[tiab] AND hardness[tiab]))	546886
4	((“Quasi-experimental studies”(pt) OR "randomized controlled trial"[pt] OR "controlled clinical trial"[pt] OR "randomized"[tiab] OR "randomised"[tiab] OR "randomization"[tiab] OR "randomisation"[tiab] OR	5950820

Search	Results	Search type
	"placebo"[tiab] OR "drug therapy"[sh] OR "randomly"[tiab] OR "trial"[tiab] OR "groups"[tiab] OR "Clinical trial"[pt] OR “clinical trial”[tiab] OR “clinical trials”[tiab] OR "evaluation studies"[Publication Type] OR "evaluation studies as topic"[MeSH Terms] OR "evaluation study"[tiab] OR "evaluation studies"[tiab] OR "intervention studies"[MeSH Terms] OR "intervention study"[tiab] OR "intervention studies"[tiab] OR "case-control studies"[MeSH Terms] OR "case-control"[tiab] OR "cohort studies"[MeSH Terms] OR "cohort"[tiab] OR "longitudinal studies"[MeSH Terms] OR "longitudinal”[tiab] OR "longitudinally"[tiab] OR "prospective"[tiab] OR "prospectively"[tiab] OR "retrospective studies"[MeSH Terms] OR "retrospective"[tiab] OR "follow up"[tiab] OR "comparative study"[Publication Type] OR "comparative study"[tiab] OR "systematic"[subset] OR "meta-analysis"[Publication Type] OR "meta-analysis as topic"[MeSH Terms] OR "meta-analysis"[tiab] OR "meta-analyses"[tiab]) OR "cross-sectional study"[tiab] OR "cross-sectional studies"[tiab] OR "community-based survey"[tiab] OR "descriptive studies"[tiab] NOT (Editorial[ptyp] OR "Letter"[ptyp] OR "Comment"[ptyp]) NOT (animals[mh])	
5	("Central America"[Mesh] OR "Nicaragua"[tiab] OR "El Salvador"[tiab] OR "Honduras"[tiab] OR "Guatemala"[tiab] OR "Panama"[tiab] OR "Costa Rica"[tiab] OR ¨Mexico"[tiab])	17787
6	((((((("Mesoamerican Nephropathy"[Mesh] OR "mesoamerican nephropathy"[tiab] OR "Decreased Kidney Function"[tiab] OR "decreased kidney function"[tiab] OR "Chronic Kidney Disease"[tiab] OR "Chronic Kidney Disease of non-traditional"[tiab] OR "chronic kidney disease of unknown"[tiab] OR "Nefrolempa"[tiab] OR ckdu[tiab] OR ckdnt[tiab]))))) AND (("Kidney Diseases"[Mesh] OR "Chronic Kidney Disease"[tiab] OR "chronic kidney disease"[tiab] OR "chronic kidney diseases"[tiab] OR "chronic	48

Search	Results	Search type
	renal disease"[tiab] OR "chronic renal diseases"[tiab] OR ckdu[tiab]))))) AND ((("Agriculture"[Mesh] OR "Chemistry, Agricultural"[Mesh] OR "Agricultural Workers' Diseases"[Mesh] OR "Pesticides"[Mesh] OR "Pesticides" [Pharmacological Action] OR "Pesticide Residues"[Mesh] OR "Agrochemicals"[Mesh] OR "Metals, Heavy"[Mesh:NoExp] OR "Aluminum"[Mesh] OR "Fluorides"[Mesh] OR "Arsenic"[Mesh] OR "Cadmium"[Mesh] OR "Lead"[Mesh] OR "Groundwater"[Mesh] OR “agriculture"[tiab] OR “agricultural"[tiab] OR “farm"[tiab] OR “pesticide"[tiab] OR “pesticides"[tiab] OR “agrochemical"[tiab] OR “heavy metal”[tiab] OR “heavy metals”[tiab] OR "Aluminum"[tiab] OR "Fluorides"[tiab] OR "fluoride"[tiab] OR "Arsenic"[tiab] OR "Cadmium"[tiab] OR “dehydration”[tiab] OR “heat stress”[tiab] OR “Heat Stress”[tiab] OR “Heat-Related”[tiab] OR “heat related”[tiab] OR “Heat exposure”[tiab] OR “Sugarcane”[tiab] OR “sugarcane”[tiab] OR “harvesters”[tiab] OR “NSAIDs”[tiab] OR "Groundwater"[tiab] OR “ground water”[tiab] OR “hard water”[tiab] OR (water[tiab] AND hardness[tiab]))))) AND (("Central America"[Mesh] OR "Nicaragua"[tiab] OR "El Salvador"[tiab] OR "Honduras"[tiab] OR "Guatemala"[tiab] OR "Panama"[tiab] OR "Costa Rica"[tiab] OR ¨Mexico"[tiab]))	

Supplementary table 2: Embase search strategy.

Search	Results	Search type
1	Kidney Diseases.af. or Chronic Kidney Disease.ab. or chronic kidney diseases.ab. or chronic kidney disease.ab. or Chronic Renal Disease.ab. or chronic renal disease.ab. or chronic renal diseases.ab. or ckdu.ab.	68314
2	Mesoamerican Nephropathy.af. or mesoamerican nephropathy.ab. or Decreased Kidney Function.ab. or decreased kidney function.ab. or Chronic Kidney Diseases.ab. or chronic kidney disease.ab. or Chronic Kidney Disease of unknown.ab. or Chronic Kidney Disease of non-traditional.ab. or Nefrolempa.ab. or ckdu.ab. or ckdnt.ab.	43475
3	(Agriculture.af. or Chemistry.ab. or Agricultural.ab. or Agricultural Workers Diseases.ab. or Pesticides.ab. or Pesticide residues.ab. or Agrochemicals.ab. or Metals, Heavy.ab. or Aluminum.ab. or Fluorides.ab. or Arsenic.ab. or Lead.ab. or Groundwater.ab. or agriculture.ab. or agricultural.ab. or farm.ab. or pesticide.ab. or pesticides.ab. or agrochemical.ab. or heavy metal.ab. or heavy metals.ab. or fluorides.ab. or fluoride.ab. or dehydration.ab. or heat stress.ab. or Heat Stress.ab. or Heat-Related.ab. or heat related.ab. or Heat exposure.ab. or Sugarcane.ab. or sugarcane.ab. or harvesters.ab. or NSAIDs.ab. or Groundwater.ab. or ground water.ab.) and hardness water.ab.	6
4	Central America.af. or Nicaragua.ab. or El Salvador.ab. or Honduras.ab. or Costa Rica.ab. or Guatemala.ab. or Panama.ab. or Mexico.ab. or Mesoamerica.ab.	64088
5	(quasi-experimental studies.af. or randomized controlled trial.af. or controlled clinical trial.ab. or randomized.ab. or randomised.ab. or randomization.ab. or randomisation.ab. or placebo.ab. or drug therapy.ab. or Trial.ab. or	2232648

Search	Results	Search type
	clinical trials.ab. or evaluation studies as topic.ab. or case-control.ab. or longitudinal studies.ab. or prospective.ab. or retrospective.ab. or meta-analyses.ab. or cross-sectional studies.ab. or cross-sectional study.ab. or community-based survey study.ab. or intervention studies.ab. or intervention study.ab.) not Editorial Letter.ab. not Comment.af. not Animal.af.	
6	limit 1 to (adolescents and adults human and Spanish and English language)	51828
7	1 and 2 and 6	63

Supplementary table 3: Web of Science search strategy.

Search	Results	Search type
1	(TS=(¨Kidney Diseases¨ OR ¨chronic kidney disease¨ OR ¨chronic kidney diseases¨ OR ¨chronic renal disease¨ OR ¨chronic renal diseases¨ OR ¨chronic kidney failure¨ OR ¨chronic renal failure¨ OR ¨ckdu¨) AND CU=("Nicaragua" OR "Honduras" OR "Costa Rica" OR "Panama" OR "Guatemala" OR "El Salvador" OR "Mexico")) *AND* **LANGUAGE:** (English)	661
2	(TS=(¨mesoamerican nephropathy¨ OR ¨decreased kidney function¨ OR ¨decreased kidney function of unknown¨ OR decreased kidney function of non-traditional¨ OR ¨CKDu¨ OR ¨CKDnt¨) AND CU=(¨Nicaragua¨ OR ¨Costa Rica¨ OR ¨Guatemala¨ OR ¨Honduras¨ OR ¨Mexico¨ OR ¨Ël Salvador¨)) *AND* **LANGUAGE:** (English)	117
3	(TS=("Agriculture" OR "Agricultural" OR "Pesticides" OR "Pesticides" OR "Agrochemicals" OR "Heat stress" OR "heat stress" OR "sugarcane workers" OR "dehydration" OR "heavy metals" OR "heavy metal" OR "Aluminum" OR "Fluoride" OR "fluorides" OR "Arsenic" OR "Cadmium" OR "Groundwater" OR "ground water" OR "hard water" OR (water AND "hardness")) AND CU=("Nicaragua" OR "Honduras" OR "Costa Rica" OR "Panama" OR "Guatemala" OR "El Salvador" OR "Mexico")) *AND* **LANGUAGE:** (English)	8630
4	(TS=("risk" OR "risks" OR "epidemiology" OR "adverse effects" OR "harm" OR "exposure" OR "exposed" OR "aetiology") AND CU=("Nicaragua" OR "Honduras" OR "Costa Rica" OR "Panama" OR "Guatemala" OR "El Salvador" OR "Mexico")) *AND* **LANGUAGE:** (English)	18142

Search	Results	Search type
5	(TS=("Intervention studies" OR "intervention study" OR "intervention studies" OR "Randomized" OR "randomised" OR "randomization" OR "randomisation" OR "placebo" OR "randomly" OR "trial" OR "groups" OR "Clinical trial" OR "clinical trials" OR "evaluation study" OR "evaluation studies" OR "case-control" OR "cohort" OR "longitudinal" OR "longitudinally" OR "prospective" OR "prospectively" OR "retrospective" OR "follow up" OR "comparative study" OR "systematic review" OR "meta-analysis" OR "metaanalysis" OR "cross sectional study" OR "survey study" OR "Cross-sectional studies" OR "cross sectional survey" OR "intervention study" OR "intervention studies") AND CU=("Nicaragua" OR "Honduras" OR "Costa Rica" OR "Panama" OR "Guatemala" OR "El Salvador" OR "Mexico")) *AND* **LANGUAGE:** (English and Spanish)	25011
6	#1 AND #2 AND #3 AND #4 AND #5	10
7	#1 AND #2 AND #3 AND #4	20

Supplementary table 4: Inclusion and exclusion criteria for defining study eligibility

Items	*Included*	*Excluded*
Participants	Adolescent and adult human participants	Animal studies
Study settings	Population, community setting or occupational workers from Mesoamerica	
Exposure of interest	• Heat stress • Dehydration • Pesticides • NSAIDs • Occupational exposure • Environmental toxin (heavy metals, silica, etc.) • Infectious diseases	Traditional risk factors: • Hypertension • Diabetes • Glomerulonephritis
Outcomes of interest	Reduced estimated glomerular filtration rate, elevated serum creatinine levels or chronic kidney disease of undetermined cause	
Study methodology	Cross-sectional studies, case-control studies, cohort studies reporting original data.	Case reports, editorial letters, case series Descriptive studies without a comparison group.
Publication details	Any publication date Language: English and Spanish	Articles published in other languages, such as German, Chinese or Portuguese

Supplementary table 5: Risk factors for CKDu (eGFR<60 mL/min) reported in epidemiological studies in Mesoamerica

Authors	Country	Risk factors associated reported in epidemiological studies																					Urinary biomarkers of kidney function
		Age	Sex	Education level	Occupations							Altitude	Hypertension	Diabetes	NSAIDs	Heat stress	Heavy metal	Water intake	Alcohol	Pesticide exposure	Bolus (Isotonic solutions) at work	Hyperuricaemia	Family history of CKDu	
			(Male vs Female)	(Low vs High)	Sugarcane	Agriculture	Cotton	Mining	Coffee	Construction	Reference occupation	(Low vs High)	(Yes vs No)	(Yes vs No)	(Yes vs No)	(Yes vs No)	(High vs Normal)	(High vs Low)	(Yes vs No)	(Apply vs No apply)	(Yes vs No)	(Yes vs No)	(Yes vs No)	
Cohort studies																								
Wesseling C, et al (2016)[23]	Nicaragua	Yes	Male	Yes	Yes	NA	NA	NA	NA	NA	Administrative work	NR	No	No	No	Yes	NR	NR	NR	NR	NR	Yes	NR	NGAL, KIM-1, Hsp
Laws R, et al (2016)[26]	Nicaragua	Yes	Male	NR	Yes	NA	NA	NA	NA	NA	Factory work	NR	NR	NR	NR	Yes	NR	No	NR	Yes	No	NR	NR	NGAL, IL-18, NAG
Laws R, et al (2015)[45]	Nicaragua	Yes	Male	NR	Yes	NA	NA	NA	NA	NA	Factory work	NR	NR	NR	NR	Yes	NR	Yes	NR	NR	No	NR	NR	Not studied
Cross-sectional studies																								
Kupferman J, et al (2016)[27]	Nicaragua	Yes	Male	NR	No	NR	NR	NR	NR	NR	No past or current work in the sugarcane industry	NR	Yes	No	No	NR	NR	NR	NR	NR	NR	NR	NR	Not studied
Wesseling C, et al (2016)[24]	Nicaragua	Yes	Male	Yes	Yes	Yes	NR	NR	NR	Yes	Agriculture	NR	No	No	No	Yes	NR	Yes	No	Yes	Yes	Yes	NR	Not studied
Lebov J, et al (2015)[30]	Nicaragua	Yes	Male	Yes	NR	NR	NR	NR	NR	NR	NR	NR	Yes	No	NR	NR	NR	Yes	Yes	NR	NR	NR	No	Not studied
Laux T, et al (2012)[31]	Nicaragua	Yes	Female	NR	NA	Yes	NA	NA	No	NA	Economically inactive population	NA	No	Yes	No	NR	NR	NR	NR	No	NR	NR	NR	Not studied
Torres C, et al (2010)[15]	Nicaragua	Yes	Male	NR	Yes	Yes	NR	Yes	NR	NR	Coffee/fishing and service	Yes	Yes	No	No	NR	NR	NR	No	NR	NR	NR	NR	Not studied
González-Quiroz M. (2010)[110]	Nicaragua	Yes	Male	NR	Yes	Yes	NR	NA	NR	NR	Yes vs no	NR	Yes	No	No	Yes	NR	Yes	No	No	NR	NR	NR	Not studied
Garcia-Trabanino R, et al (2015)[22]	El Salvador	Yes	Male	NR	Only	NA	NA	NA	NA	NA	Only sugarcane work	Yes	No	No	No	Yes	NR	Yes	No	No	NR	Yes	NR	NAG
Orantes C, et al (2014)[32]	El Salvador	Yes	Male	NR	NR	Yes	NR	NR	NR	NR	Non-agricultural work	NR	No	No	No	NR	NR	NR	NR	No	NR	NR	No	Not studied
Peraza S, et al (2012)[18]	El Salvador	Yes	Male	NR	NR	Yes	Yes	NA	No	No	Never worked in agriculture	Yes	No	No	No	NR	NR	NR	No	NR	NR	NR	NR	Not studied
Orantes C, et al (2011)[20]	El Salvador	Yes	Male	NR	NR	No	NR	NR	NR	NR	Non-agricultural work	NR	Yes	No	No	NR	NR	NR	No	No	NR	NR	Yes	Not studied

Authors	Country	Risk factors associated reported in epidemiological studies																						Urinary biomarkers of kidney function
		Age	Sex	Education level	Occupations							Altitude	Hypertension	Diabetes	NSAIDs	Heat stress	Heavy metal	Water intake	Alcohol	Pesticide exposure	Bolus (Isotonic solutions) at work	Hyperuricaemia	Family history of CKDu	
			(Male vs Female)	(Low vs High)	Sugarcane	Agriculture	Cotton	Mining	Coffee	Construction	Reference occupation	(Low vs High)	(Yes vs No)	(Yes vs No)	(Yes vs No)	(Yes vs No)	(High vs Normal)	(High vs Low)	(Yes vs No)	(Apply vs No apply)	(Yes vs No)	(Yes vs No)	(Yes vs No)	
Garcia-Trabanino R, et al (2005)[111]	El Salvador	Yes	Male	NR	NR	Yes	NR	NR	NR	NR	Non-agricultural work	Yes	Yes	Yes	NR	NR	NR	NR	No	No	NR	NR	NR	Not studied
Case-control studies																								
Raines N, et al (2014)[21]	Nicaragua	Yes	Male	NR	Yes	Yes	NR	NR	NR	NR	No sugarcane work or agricultural work	Yes	No	No	No	NR	NR	No	No	Yes	Yes	NR	NR	Not studied
O'Donnell J, et al (2010)[19]	Nicaragua	Yes	Male	No	No	No	No	NA	NA	NR	No agricultural work, cotton work or sugarcane work	Yes	Yes	No	No	No	No	No	No	No	NR	NR	NR	Not studied
Sanoff S, et al (2010)[69]	Nicaragua	Yes	Male	NR	No	Yes	No	NA	NR	NR	No agricultural work or sugar mill work	NR	No	No	NR	NR	NR	Yes	Yes	No	NR	NR	No	Not studied
Ecological studies																								
Wesseling C, et al (2015)[106]	Costa Rica	Yes	Male	NA	Yes	Yes	NA	NA	NA	NA	NA	NA	NA	NA	NA	Environmental Temperature	NA	NA	NA	NA	NA	NA	NA	NA
Laux T, et al (2015)[112]	Guatemala	Yes	Male	NA	Yes	NR	NA	NA	NA	NA	NA	NA	NA	NA	NA	Environmental Temperature	NA	NA	NA	NA	NA	NA	NA	NA
VanDervort D, et al (2014)[113]	El Salvador	NR	NR	NA	Yes	Yes	Yes	NR	No	NA	NA	NR	NA	NA	NA	Environmental Temperature	NA	NA	NA	NA	NA	NA	NA	NA

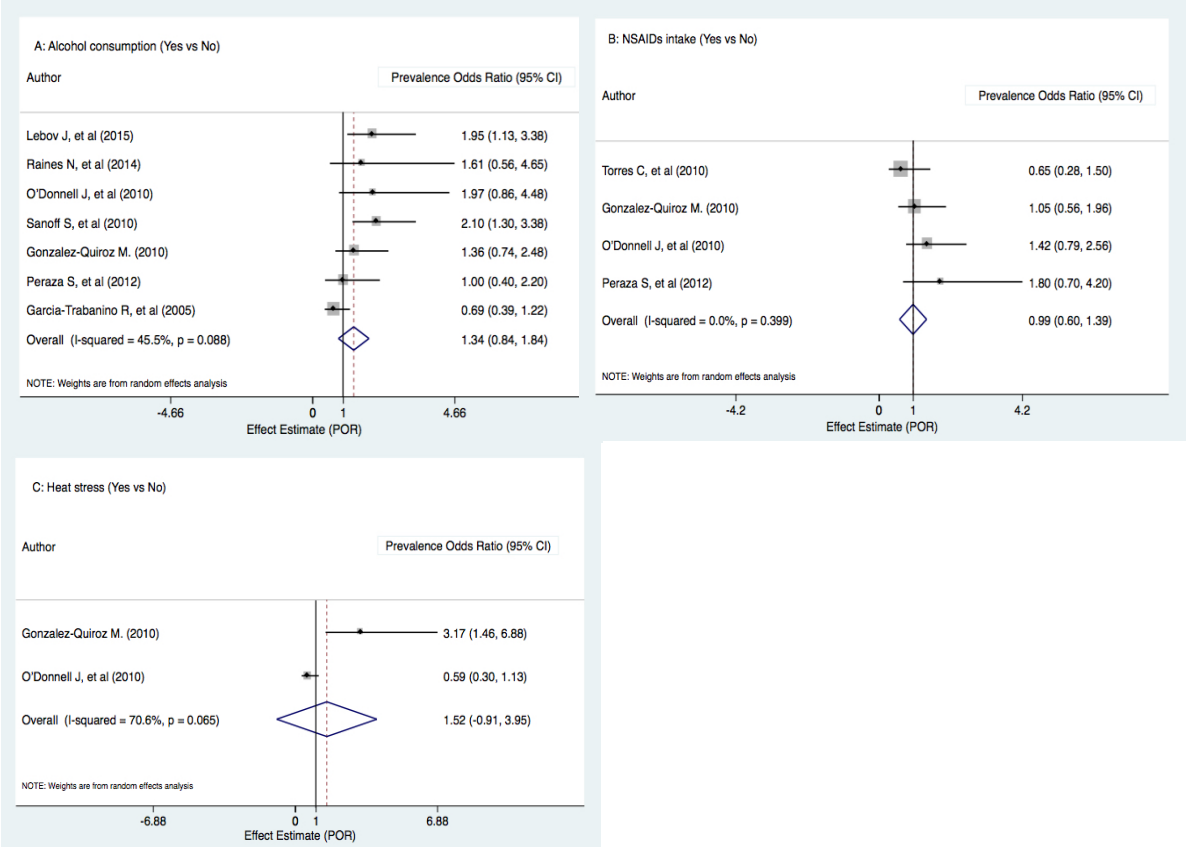
Risk factors associated with CKDu

Risk factors not associated with CKDu

Reference group for occupation

Risk factors that were not studied or reported

Supplementary figure 1: Forest plots of associations of alcohol consumption (A), non-steroidal anti-inflammatory drug use (B) and heat stress (C) estimates with chronic kidney disease of undetermined caused (eGFR<60 mL/min)



Black diamond data markers represent PORs; horizontal lines represent the 95% CIs; the size of the grey square marker indicates the statistical weight of the study using the random effects meta-analysis. A diamond data marker denotes the overall POR and 95% CI for the outcome of interest.

Abbreviations: *NSAIDs: non-steroidal anti-inflammatory drugs*

Supplementary table 6: Quality assessment of occupational studies including the rationale (n=5)

Author	Study design	Selection bias	Loss to follow-up	Non-differential misclassification exposure	Information bias of exposure	Non-differential misclassification of outcome	Information bias of outcome	Confounding	Reverse causation
Wesseling C, et al (2016)[24]	Cross-sectional	Low: High participation rate (86%) in all occupations	N/A: Cross-sectional study	Medium: water intake, self-report of nephrolithiasis, self-reports of urinary tract infection and use of NSAIDs	Medium: The retrospective assessment of some exposures was prone to recall bias, but a standardized questionnaire was administered by well-trained interviewers and a certain number of questionnaires were selected for re-testing by a fieldwork assistant to confirm the information.	Low: The use of the CKD-EPI equation has not been validated in the Nicaraguan population.	High: The use of a non-standard eGFR threshold for determining the outcome (eGFR <80 mL/min/1.73 m^2) and a single measure of eGFR were only prone to misclassification of AKI.	Medium: Restricted to sugarcane cutters, but not adjusted for age	High: Some exposures such as water intake, urinary tract infection and the use of NSAIDs, may be prone to reverse causation.
Wesseling C, et al (2016)[23]	Cohort (Changes in GFR across the harvest period)	Low: High participation rate (88%) at baseline (1104 of 1249 workers sampled at pre/harvest screens)	High: 50% of enrolled participants were fired at the middle of the harvest	Medium: water intake, self-report of nephrolithiasis, diabetes and use of NSAIDs	Low: Exposure data were collected prospectively and a standardized questionnaire was administered by well-trained interviewers; a certain number of questionnaires were selected for re-testing by a fieldwork assistant to confirm the information.	Low: The use of the CKD-EPI equation has not been validated in the Nicaraguan population.	Low: Kidney function was measured prospectively during the harvest season.	Medium: Adjusted for sex and age, but not for other known risk factors for CKD	High: Some exposures, such as water intake, nephrolithiasis, and the use of NSAIDs, may be prone to reverse causation.
Laws R, et al (2016)[26]	Cohort (Changes in GFR across the harvest period)	Low: High participation rate (88%) at baseline (1104 of 1249 workers sampled at pre/harvest screens)	High: 55% of sugarcane workers were to lost follow up at late harvest for many reasons: stopped working, moved to different regions, and financial and logistical constraints	Medium: self-report of water and sugar intake	Low: Exposure data were collected prospectively.	Low: The use of the CKD-EPI equation has not been validated in the Nicaraguan population.	Low: Kidney function and urinary biomarkers were measured prospectively before and during late harvest.	Medium: Adjusted for sex, age, and years worked at the company, but not for other known risk factors for CKD	High: Some exposures, such as water intake, may be prone to reverse causation.

Author	Study design	Selection bias	Loss to follow-up	Non-differential misclassification exposure	Information bias of exposure	Non-differential misclassification of outcome	Information bias of outcome	Confounding	Reverse causation
Laws R, et al (2015)[45]	Cohort (Changes in GFR across the harvest period)	Low: High participation rate (88%) at baseline	High: 55% of sugarcane workers were lost to follow-up at late harvest for many reasons: stopped working, moved to different regions, and financial and logistical constraints	Medium: self-report of water and electrolyte solution intake before and during late harvest	Low: Exposure data were collected prospectively.	Low: The use of the CKD-EPI equation has not been validated in the Nicaraguan population.	Low: Kidney function was measured prospectively before and during late harvest.	Medium: Adjusted for sex, age, and years worked at the company, but not for other known risk factors for CKD	High: Some exposures, such as water intake, may be prone to reverse causation.
Garcia-Trabanino R, et al (2015)[22]	Cohort (Changes in GFR across shifts)	Low: High participation rate (84%) among sugarcane workers	N/A: Cross-sectional study	Medium: self-report of nephrolithiasis, diabetes, use of NSAIDs, diuretics, and sweet drinks	Medium: Retrospective assessments of some exposures are prone to recall bias, but a standardized questionnaire was administered by well-trained interviewers, and a certain number of questionnaires were selected for re-testing by a fieldwork assistant to confirm the information.	Low: The use of the CKD-EPI equation has not been validated in the Nicaraguan population.	Low: Two measures of eGFR across shifts were only prone to detect AKI.	High: Unadjusted for known risk factors for CKD	High: Some exposures, such as nephrolithiasis and the use of NSAIDs, may be prone to reverse causation.

Abbreviations: NSAIDs; non-steroidal anti-inflammatory drugs, CKD; chronic kidney disease, N/A; not applicable, N/R: not reported, eGFR; estimated glomerular filtration rate, AKI; acute kidney injury.

Supplementary table 7: Quality assessment of community-based studies including the rationale (n=20)

Author	Study design	Selection bias	Loss to follow-up	Non-differential misclassification exposure	Information bias of exposure	Non-differential misclassification of outcome	Information bias of outcome	Confounding	Reverse causation
González M, et al (2017)[114]	Cohort	Low: High participation rate (97%) for young adults aged between 18 to 30 years.	N/R: Only baseline values were reported.	Medium: self-reported labour history and NSAID use or water intake	Medium: Retrospective assessments of some exposures are prone to recall bias. However, a standardized questionnaire was administered by well-trained interviewers, and a certain number of questionnaires were selected for re-testing by a fieldwork assistant to confirm the .information	Low: The use of the CKD-EPI equation has not been validated in the Nicaraguan population.	High: Arbitrary cut-off point for eGFR <90 mL/min and a single measure of eGFR were only prone to misclassification of AKI.	Medium: Stratified by sex. Participants with diabetes or hypertension were excluded from the study.	High: Some exposures, such as labour history, NSAID use, and water intake, are prone to reverse causation.
Minnings K, et al (2016)[115]	Cross-sectional study with a selected follow-up	Medium: 75% of response participation	High: Only those with low eGFR were followed-up	Medium: self-reported labour and medical history	Medium: Retrospective assessments of some exposures are prone to recall bias.	Low: Use of the non-validated MDRD equation for the Nicaraguan population.	High: Kidney function was determined based on a creatinine level of 1.5 mg/dL for males and 1.2 mg/dL for females or an eGFR <60 mL/min/1.73 m^2. However, a single measure of eGFR was only prone to misclassification of AKI	High: Unadjusted for known risk factors for CKD	High: Some exposures, such as labour history, HTN and nephrolithiasis, are prone to reverse causation.
Kupferman J, et al (2016)[27]	Cross-sectional	Low: All participants were recruited from families with CKDu in the community.	N/A: Cross-sectional study	Medium: self-report of diabetes, past or present work in the sugarcane industry and NSAID use	Medium: Retrospective assessments of some exposures are prone to recall bias, and all questionnaires were reviewed in the field.	Low: The use of the CKD-EPI equation has not been validated in the Nicaraguan population.	Low: The diagnosis of CKDu was re-confirmed by determining serum creatinine levels.	Medium: Adjusted for eGFR, age and use of uric acid medication	High: Some exposures, such as past or present work in the sugarcane industry and NSAID use, are prone to reverse causation.
Lebov J, et al (2015)[30]	Cross-sectional	Low: The participation rate was 91%. Participants aged between 18 to 70 years old were selected from a surveillance system based on demography.	N/A: Cross-sectional study	Medium: self-report of diabetes, blood pressure and water intake	Medium: Retrospective assessments of some exposures are prone to recall bias. However, interviewers were trained, and a fieldwork assistant reviewed the questionnaires in the field.	Low: Use of the non-validated MDRD equation for the Nicaraguan population	Medium: The single measure of eGFR was only prone to misclassification of AKI.	Low: Adjusted for age, sex, diabetes, and high blood pressure.	High: Some exposures, such as HTN and water intake, are prone to reverse causation.

Author	Study design	Selection bias	Loss to follow-up	Non-differential misclassification exposure	Information bias of exposure	Non-differential misclassification of outcome	Information bias of outcome	Confounding	Reverse causation
Raines N, et al (2014)[21]	Cross-sectional/nested case-control	Medium: The participation rate was 62% among people aged 15 to 69 years and controls were selected from participants of a cross-sectional study.	N/A: Cross-sectional study	Medium: self-reported water intake, sugar intake, NSAID use, and pesticide exposure	Medium: Retrospective assessments of some exposures are prone to recall bias. However, interviewers were trained, and the questionnaire was validated prior data collection. All equipment was calibrated daily.	Low: The use of the CKD-EPI equation has not been validated in the Nicaraguan population.	Medium: A single measure of eGFR is only prone to misclassification of AKI	Medium: Adjusted for sex and age, but not for other known risk factors for CKD	High: Some exposures, such as water intake, NSAID use, and pesticide exposure, are prone to reverse causation.
Laux T, et al (2012)[31]	Cross-sectional	Low: All participants between 20 to 60 years were recruited at community level and participation rate was 90%.	N/A: Cross-sectional study	Medium: self-report of diabetes, and history of nephrotoxic drug use	Medium: retrospective assessment of some exposures prone to recall bias Low: Research team conducted the interviews by applying a validated questionnaire in a similar population. Scale and sphygmomanometer were calibrated every day.	Low: Use of non-validated MDRD equation for Nicaraguan population	Medium: Single measure of eGFR only prone misclassification of AKI	Medium: Adjusted for BMI, hypertension and diabetes	High: Some exposures such as NSAID use is prone to reverse causation
O'Donnell J, et al (2010)[19]	Cross-sectional/nested case-control	Low: Participants over 18 years were recruited at community level and the participation rate was 91%. Controls were randomly selected from participants of a cross-sectional study	N/A: Cross-sectional study	Medium: self-report of diabetes, labour history, NSAIDs and antibiotic use	Medium: retrospective assessment of some exposures prone to recall bias and research assistant applied a standardized questionnaires and calibrated instruments (scale and sphygmomanometer). Training of interviewers.	Low: Use of non-validated MDRD equation for Nicaraguan population	Medium: Single measure of eGFR only prone misclassification of AKI and Serum creatinine level was determined by StatSensor device and re-analysed by using an IDMS-standardized creatinine assays	Medium: Adjusted for sex and age but not for other known risk factors for CKD	High: Some exposures such as labour history, NSAIDs use are prone to reverse causation
Sanoff S, et al (2010)[69]	Case-control	High: Controls were selected from groups of volunteers with high history of alcohol consumption. Participants rate was 99%	N/A: Cross-sectional study	Medium: self-report of diabetes, occupational history, and water consumption.	High: Participants were selected from a groups high level of exposure and retrospective assessment of some exposures prone to recall bias	Low: Use of non-validated MDRD equation.	Medium: Single measure of eGFR only prone misclassification of AKI	Low: Adjusted for age, gender, hypertension, a reported history of diabetes, a family history of end stage kidney disease, and body mass index.	High: Some exposures such as labour history and NSAIDs use are prone to reverse causation

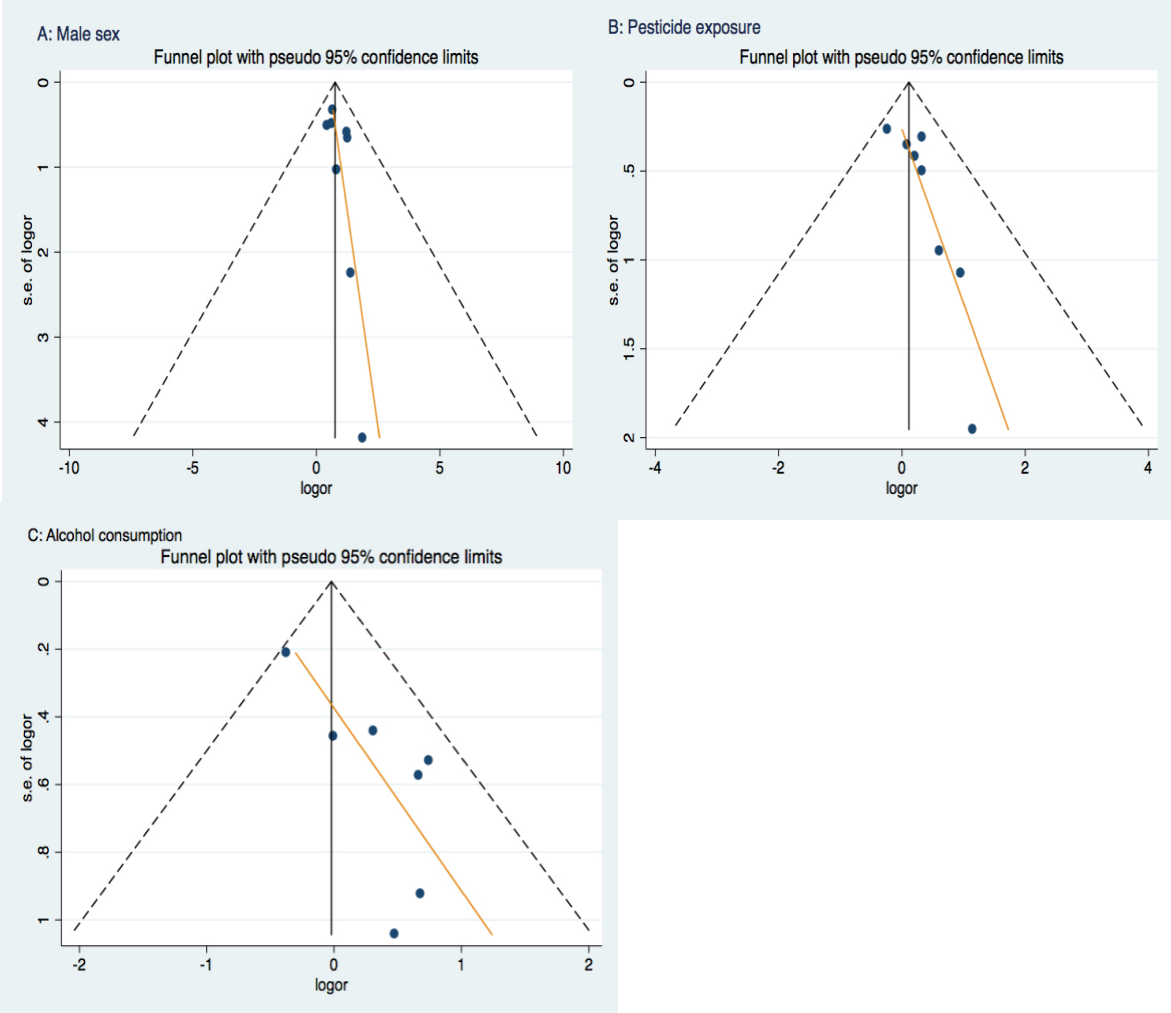
Author	Study design	Selection bias	Loss to follow-up	Non-differential misclassification exposure	Information bias of exposure	Non-differential misclassification of outcome	Information bias of outcome	Confounding	Reverse causation
Torres C, et al (2010)[15]	Cross-sectional	Low: Participants between 20 to 60 years were recruited at community level and the Participation rate was 83%.	N/A: Cross-sectional study	Medium: self-report of diabetes and self-reported of urinary tract infection.	Medium: retrospective assessment of some exposures prone to recall bias	Low: Use of CKD-EPI equation which has not been validated in the Nicaraguan population	Medium: Single measure of eGFR only prone misclassification of AKI	Low: Adjusted for age, sex, hypertension, obesity and diabetes	High: Some exposures such as self-report of urinary tract infection are prone to reverse causation
González-Quiroz M. (2010)[110]	Cross-sectional	Low: Participants between 20 to 60 years were recruited by random selection sample and the Participation rate was 97%.	N/A: Cross-sectional study	Medium: self-report of labour history, water-intake, diabetes, nephrolithiasis and urinary tract infection	Medium: retrospective assessment of some exposures prone to recall bias, but well training interviewers applied a standardized questionnaire. Research team reviewed all questionnaire at field	Low: Use of CKD-EPI equation which has not been validated in the Nicaraguan population	Medium: Single measure of eGFR only prone misclassification of AKI	Low: Adjusted for age, sex, hypertension, obesity and diabetes	High: Some exposures such as water intake, nephrolithiasis and occupation are prone to reverse causation
Orantes C, et al* (2016)[116]	Cross-sectional	Low: Participants under 18 years were recruited at community level and participation rate was 97.8%.	N/A: Cross-sectional study	N/R: No exposure data was reported in this study	N/R: No exposure data was reported in this study	Low: Use of non-validated Schwartz equation.	Low: Objective definition of outcome (eGFR <60 mL/min/1.73 m^2, with or without markers of kidney damage) with two measurements of serum creatinine within three months' difference proved the CKD diagnosis.	High: Unadjusted for known risk factors for CKD	N/R: No exposure data was reported in this study
Orantes C, et al* (2015)[117]	Cross-sectional	Low: Women were recruited from agriculture community	N/A: Cross-sectional study	Medium: self-report of diabetes, use of NSAIDs, and occupations.	Medium: retrospective assessment of some exposures prone to recall bias but all instruments and tools were calibrated. Measurements were done by certified personnel	Low: Use of non-validated MDRD equation.	Low: Objective definition of outcome (eGFR <60 mL/min/1.73 m^2) with two measurements of serum creatinine within three months' difference proved the CKD diagnosis	High: Unadjusted for known risk factors for CKD	High: Some exposures such as use of NSAIDs, and occupation are prone to reverse causation
Vela X, et al* (2014)[118]	Cross-sectional	Low: Participants were recruited from a farming communities and the participation rate was 91.4%	N/A: Cross-sectional study	Medium: self-reported use of NSAIDs, antibiotics, and occupations.	Medium: retrospective assessment of some exposures prone to recall bias but all instruments and tools were calibrated. Measurements were done by certified personnel	Low: Use of CKD-EPI equation which has not been validated in the Nicaraguan population	Low: Objective definition of outcome (eGFR <60 mL/min/1.73 m^2) with two measurements of serum creatinine within three months' difference proved the CKD diagnosis	High: Unadjusted for known risk factors for CKD	High: Some exposures such as use of NSAIDs and occupation are prone to reverse causation

Author	Study design	Selection bias	Loss to follow-up	Non-differential misclassification exposure	Information bias of exposure	Non-differential misclassification of outcome	Information bias of outcome	Confounding	Reverse causation
Orantes C, et al* (2014)[32]	Cross-sectional	Low: Participants were recruited at the community level and the participation rate was 100%.	N/A: Cross-sectional study	Medium: self-reported diabetes, use of NSAIDs, and occupation.	Medium: Retrospective assessments of some exposures are prone to recall bias, but all instruments and tools were calibrated. Measurements were performed by certified personnel.	Low: Use of a non-validated MDRD equation.	Low: An objective definition of outcome (eGFR <60 mL/min/1.73 m^2) with two measurements of serum creatinine levels within three months confirmed the CKD diagnosis.	Low: Adjusted for known risk factors for CKD	High: Some exposures, such as the use of NSAIDs and occupation, are prone to reverse causation.
Peraza S, et al (2012)[18]	Cross-sectional	Low: Participants aged between 20 to 60 years were recruited at the community level, and the participation rate was 77%.	N/A: Cross-sectional study	Medium: self-report of diabetes, nephrolithiasis and the use of NSAIDs	Medium: Retrospective assessments of some exposures are prone to recall bias, but a standardized questionnaires and calibrated instruments (scale and sphygmomanometer) were used.	Low: The use of the CKD-EPI equation has not been validated in the Nicaraguan population.	Medium: The single measure of eGFR is only prone to misclassification of AKI.	Low: Adjusted for age, sex, hypertension and diabetes	High: Some exposures, such as nephrolithiasis and the use of NSAIDs, are prone to reverse causation.
Orantes C, et al* (2011)[20]	Cross-sectional	Medium: Participants were recruited at the community level from individuals with a long history of pesticide use, and the participation rate was 88.3%.	N/A: Cross-sectional study	Medium: self-reported use of NSAIDs, medicinal plants, infectious diseases and occupation	Medium: Retrospective assessments of some exposures are prone to recall bias, but all instruments and tools were calibrated. Measurements were performed by certified personnel.	Low: Use of a non-validated MDRD equation.	Low: An objective definition of outcome (eGFR <60 mL/min/1.73 m^2) with two measurements of serum creatinine levels within three months confirmed the CKD diagnosis.	High: Unadjusted for known risk factors for CKD	High: Some exposures, such as the use of NSAIDs, use of medicinal plants, infectious diseases and occupation, are prone to reverse causation.
Garcia-Trabanino R, et al (2005)[111]	Cross-sectional	High: The participation rate was 29% (353 of 1220 subjects).	N/A: Cross-sectional study	Medium: self-report of medical history and occupation.	Medium: Retrospective assessments of some exposures are prone to recall bias.	Medium: Use of a cut-off serum creatinine level >1.5 mg/dl	Medium: An objective definition of outcomes (proteinuria and chronic renal failure)	High: Unadjusted for known risk factors for CKD	High: Some exposures, such as HTN and occupation, are prone to reverse causation.
Wesseling C, et al (2015)[106]	Ecological	Low: All Costa Rican provinces were included in the study.	N/A: Ecological study	Low: Determined from the National Weather Surveillance System	Low: Climate data from the National Weather Surveillance System and exposure data based on vital statistics	Medium: Low overall recognition of CKD	High: Recognition of CKD could vary by area.	High: Unadjusted for known risk factors for CKD	Low: Ecological study

Author	Study design	Selection bias	Loss to follow-up	Non-differential misclassification exposure	Information bias of exposure	Non-differential misclassification of outcome	Information bias of outcome	Confounding	Reverse causation
Laux T, et al (2015)[112]	Ecological	Low: Participants were recruited at dialysis programmes from different regions in Guatemala.	N/A: Ecological study	Low: Determined from the National Weather Surveillance System	Low: Climate data were obtained from the National Weather Surveillance System.	High: Not all patients have access to dialysis treatment.	High: Some regions have fewer dialysis units than other regions within the country.	High: Unadjusted for known risk factors for CKD	Low: Ecological study
VanDervort D, et al (2014)[113]	Ecological	Low: All regions from El Salvador were included in the study.	N/A: Ecological study	Low: Determined from an agricultural census and ambient temperature	Low: Exposure data were obtained from the Ministry of Environment and Natural Resources and the Ministry of Economy. CKD data were obtained from the National Health System.	Medium: Low overall recognition of CKD	High: Recognition of CKD could vary by area.	High: Unadjusted for known risk factors for CKD	Low: Ecological study

**Five studies from El Salvador applied the following CKD definition: persistence of renal damage markers for ≥3 months or GFR <60 mL/min.*
Abbreviations: NSAIDs; non-steroidal anti-inflammatory drugs, CKDu; chronic kidney disease of undetermined cause, CKD; chronic kidney disease, N/A; not applicable, N/R: not reported, eGFR; estimated glomerular filtration rate, AKI; acute kidney injury.

Supplementary figure 2: Funnel plot showing the associations between male sex, pesticide exposure and alcohol consumption with CKDu (eGFR<60 mL/min) using the log OR and its standard error (n=8)



Chapter 3. Rationale and baseline findings from a community-based longitudinal prospective cohort study among young, apparently healthy participants

3.1 Introduction to paper II

This paper was published in BioMed Central Nephrology Journal (BMC Nephrology) and presents the rationale, description and baseline findings of a community-based prospective cohort study of kidney function among the young rural population of Northwest Nicaragua.

This chapter presents and discusses the rationale and baseline results from a prospective cohort study designed to assess the sociodemographic characteristics, occupational and non-occupational exposures, lifestyle, dehydration symptoms, etc. The primary outcome was to describe features of a population of young adults without known kidney disease living in communities with a high prevalence of CKDu in Nicaragua.

Although participants with a previous diagnosis of CKD were excluded, the prevalence of potentially reduced kidney function (eGFR <90 mL/min/1.73 m^2) was 11% among males and 1% among females. Of participants with an eGFR<90 mL/min/1.73 m^2, 15% reported only working in agriculture, 10% in sugarcane only and 12% in sugarcane and any other work, including agriculture. In summary, this study recruited young adults without known kidney function impairments and without any conventional risk factors for CKD (diabetes and hypertension) to investigate the natural history of CKDu.

The baseline questionnaire and tables that describes the sociodemographic characteristics and potential factors for heat stress were included in this paper as supplementary materials. The baseline questionnaire is included in this chapter as appendix B.

3.2 Research paper cover sheet

RESEARCH PAPER COVER SHEET

PLEASE NOTE THAT A COVER SHEET MUST BE COMPLETED <u>FOR EACH</u> RESEARCH PAPER INCLUDED IN A THESIS.

SECTION A – Student Details

Student	Marvin Gonzalez-Quiroz
Principal Supervisor	Dorothea Nitsch
Thesis Title	Occupational kidney disease among young populations in northwest Nicaragua

<u>If the Research Paper has previously been published please complete Section B, if not please move to Section C</u>

<u>SECTION B – Paper already published</u>

Where was the work published?	BioMedical Central Nephrology (BMC nephrology)		
When was the work published?	2017		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	No		
Have you retained the copyright for the work?*	**Yes**	Was the work subject to academic peer review?	**Yes**

**If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.*

<u>SECTION C – Prepared for publication, but not to date published</u>

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	
Stage of publication	Choose an item.

<u>SECTION D – Multi-authored work</u>

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	The proposal for this cohort study was developed and written by Professor Neil Pearce, Dr. Ben Caplin and Professor Dorothea Nitsch. They obtained the funding for the study and the ethical approval. I developed the study design from the original proposal under the supervision of D. Nitsch, Neil Pearce and Ben Caplin. I was responsible for the community engagement, and I coordinated the fieldwork, entered data and shipped the samples to the UK. I conducted the data analysis and drafted the manuscript according to the advice of my supervisor and co-authors. The manuscript was peer-reviewed, and I edited the draft in response to suggestions from reviewers into the final version.

Student Signature: [redacted] **Date:** October 7/2018

Supervisor Signature: [redacted] **Date:** October 7/2018

3.3 Evidence of copyright retention

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3.4 Research paper cover sheet: *Rationale, description and baseline findings of a community-based prospective cohort study of kidney function amongst the young rural population of Northwest Nicaragua.*

González-Quiroz et al. BMC Nephrology (2017) 18:16
DOI 10.1186/s12882-016-0422-4

BMC Nephrology

RESEARCH ARTICLE Open Access

Rationale, description and baseline findings of a community-based prospective cohort study of kidney function amongst the young rural population of Northwest Nicaragua

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Abstract

Background: An epidemic of Mesoamerican Nephropathy (MeN) is killing thousands of agricultural workers along the Pacific coast of Central America, but the natural history and aetiology of the disease remain poorly understood. We have recently commenced a community-based longitudinal study to investigate Chronic Kidney Disease (CKD) in Nicaragua. Although logistically challenging, study designs of this type have the potential to provide important insights that other study designs cannot. In this paper we discuss the rationale for conducting this study and summarize the findings of the baseline visit.

Methods: The baseline visit of the community-based cohort study was conducted in 9 communities in the North Western Nicaragua in October and November 2014. All of the young men, and a random sample of young women (aged 18–30) without a pre-existing diagnosis of CKD were invited to participate. Glomerular filtration rate (eGFR) was estimated with CKD-EPI equation, along with clinical measurements, questionnaires, biological and environmental samples to evaluate participants' exposures to proposed risk factors for MeN.

Results: We identified 520 young adults (286 males and 234 females) in the 9 different communities. Of these, 16 males with self-reported CKD and 5 females with diagnoses of either diabetes or hypertension were excluded from the study population. All remaining 270 men and 90 women, selected at random, were then invited to participate in the study; 350 (97%) agreed to participate. At baseline, 29 (11%) men and 1 (1%) woman had an eGFR <90 mL/min/1.73 m^2.

Conclusion: Conducting a community based study of this type requires active the involvement of communities and commitment from local leaders. Furthermore, a research team with strong links to the area and broad understanding of the context of the problem being studied is essential. The key findings will arise from follow-up, but it is striking that 5% of males under aged 30 had to be excluded because of pre-existing kidney disease, and that despite doing so 11% of males had an eGFR <90 mL/min/1.73 m^2 at baseline.

Keywords: CKDu, Mesoamerican nephropathy, Follow-up, Community-based, Nicaragua

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Full list of author information is available at the end of the article

Background

There is an ongoing epidemic of chronic kidney disease of undetermined cause (CKDu) in the lowlands of the Pacific coast of Central America [1, 2]. CKDu, also termed Mesoamerican Nephropathy (MeN), is responsible for the deaths of thousands of young male adults, especially sugarcane cutters at relatively young ages [3, 4]. This apparently new clinical entity accounts for a considerable health and economic burden, both for the families and local health systems, which do not have the capacity to cope with this epidemic.

The cause(s) of MeN are not fully understood but sugarcane workers appear to be the occupational group most at risk [3, 5]. Heat stress and recurrent volume depletion are currently thought to play a key role in the evolution of the disease although investigators have also suggested toxins, infections and genetics may play a part. The evidence for and against the various factors causing the disease are reviewed elsewhere [6, 7].

In view of the substantial public health impact of this disease, and the current gaps in understanding of the potential causes and contributing factors, we designed a community based cohort study with the aim of investigating the causes and natural history of the disease [8].

Rationale for the study design

Dealing with recall bias and reverse causation

Associations reported in existing cross-sectional and case-control studies may be subject to both recall bias and reverse causation. Recall bias can be minimised by ascertaining data on exposures at baseline as part of a longitudinal study. Furthermore, dealing with reverse causation is likely to be particularly important in untangling the causes of CKDu as markers of exposure status may themselves be affected by kidney dysfunction. For example, the reported relationship between increased water consumption and CKDu [9, 10] might be explained by a failure of urinary concentration by the diseased kidney rather than water consumption actually leading to disease. A prospective study of risk factors and changes in eGFR over time in a young population with preserved kidney function at baseline will overcome this problem.

Setting

A community-based cohort represents the entire 'at-risk' population. In our study, workers from all occupations - both men and women – were eligible for recruitment. Furthermore, loss to follow-up over time, although still a significant concern, was likely to be less challenging in community-based follow-up studies when compared to occupational cohorts. This is particularly true in northwest Nicaragua where sugarcane workers can lose their job if they are found to be suffering from kidney disease and therefore the potential for loss to follow up in occupational studies is elevated [11]. Although a potential disadvantage of community-based studies is that they typically need to recruit large numbers of people if disease prevalence is low, this is less of an issue in our study given the epidemic proportions of CKDu in the region. One disadvantage of the community setting is that although we can ask questions on occupational exposures we are unable to directly quantify work related variables such as occupational heat stress.

Evaluating kidney dysfunction

A major challenge in epidemiological research of kidney disease is the accurate measurement of kidney function. Measuring the true GFR is not feasible in large studies. Furthermore there is considerable within-person and more particularly between-person measurement error when using eGFR based in the serum creatinine (SCr) level due to factors such as muscle mass, diet, exertion and hydration status. This makes studies based on comparing one-off eGFR measurement difficult to interpret. Therefore, we chose to measure within-person change in eGFR, which is inherently independent of between-person factors. Assuming that the main drivers of the within-person measurement error in the eGFR are constant, and if calculated across a number of time points, then eGFR decline at the level of the individual will be less affected by factors that are not related to the progressive kidney damage of interest [12–14].

Methods

Design

This is a community-based cohort study with total of 5 study visits planned over 2 years.

Setting

The study communities are in the Departments of León and Chinandega, in northwest Nicaragua, a region with the highest mortality rates of young populations due to ESRD [2, 4]. This area is characterised by sugarcane cultivation (70% of cultivated land), subsistence farming (beans, corn) (20%) and banana growing (10%) and the main employment source is work in sugar mill plantation during the harvest and pre-harvest.

Sample size calculations

Given that people who will develop MeN in their 30s are likely to experience a loss of at least 40 mL/min GFR over their working lives, our study aims to investigate the likely causes and natural history of the disease by quantifying early decline in eGFR and capturing data on associated exposures. The study was powered to detect exposures associated with decline in eGFR >5 mL/min/

year. We estimated that a minimum of 180 subjects would be required to achieve a power of 90% to detect an association with a binary exposure (detected on questionnaire) in 20% of the population associated with the above effect size (at $\alpha = 0.01$). Additionally, we assumed up to 20% loss to follow-up and the need to consider testing associations with multiple exposures, our aim was to recruit and follow 300 participants.

Community engagement

We first visited the community leaders to gain an understanding of the locations, distance, and availability of the communities to be part of a two-year follow-up study. The community leaders then organized large public meetings with the target population where members of the research team explained the aims and benefits of the research project. We were also in communication (in person and by telephone) with both the community leaders and participants throughout the planning and baseline phases of the project.

Study population and recruitment strategy

The source population was healthy young people (without diabetes, hypertension or CKD diagnosis by self-report) aged between 18 and 30 years living in nine communities in northwest Nicaragua (six communities in Chinandega and three in León Department).

During August to October 2014, a population census of young adults age 18 to 30 years old was performed in each community. Potential participants were asked about their medical history and those with a pre-existing diagnosis of CKD, diabetes or hypertension were excluded. As the CKDu predominantly affects males, all men eligible were invited to participate along with a random selection of eligible women, in a male to female ratio of 3:1.

Standardising the data collection methods

A team comprising the field coordinator, 10 local interviewers and two phlebotomists performed the study visits. Prior to commencing fieldwork, each team member undertook a three-day intensive training course that focused on standardising data and sample collection as well as maximising data quality.

Study visits and medical examinations

The baseline data collection in the nine communities was undertaken during October - November 2014, before the start of the sugarcane harvest. Data collection was scheduled during the working week, before the participants left for work, which meant visiting each community between 3 and 5 am. The location for data collection was a well-known public place (a church, a health care centre, a school, or the house of the community leader), to ensure ease of access for the participants and ensure that the study maintained a visible presence amongst the community. Following registration, each participant had non-invasive clinical measurements (blood pressure, height and weight) taken first, followed by blood and urine sampling. This was then followed by a detailed questionnaire administered by a trained interviewer, which was checked by re-questioning participants on a random selection of questions (Fig. 1).

Clinical measurements

For each participant, their body weight, height, blood pressure and heart rate were measured, and blood and

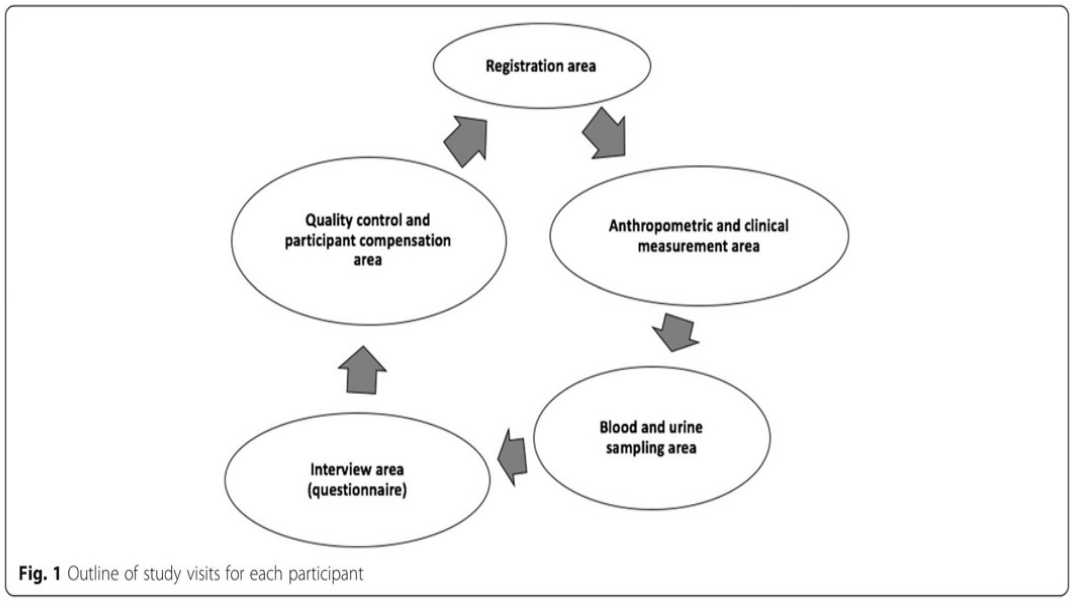

Fig. 1 Outline of study visits for each participant

urine samples were collected. Body weight was measured with minimal clothes using SECA electronic scales (Seca, Birmingham, UK). Height was measured by using a portable stadiometer (Seca, Birmingham, UK). Blood pressure and heart rate were measured in a sitting position using a calibrated digital sphygmomanometer (Omron, Kyoto, Japan) after five minutes of quiet seated rest. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg. BMI was classified into <25 kg/m^2 as normal, 25–29.9 kg/m^2 as overweight, and ≥30 kg/m^2 as obese. Those with a BMI ≥25 kg/m^2 were defined as overweight/obese.

Blood samples were collected in three vacuum tubes, two with clot activator and gel for serum separation and one with anticoagulant. These tubes were placed in an icebox (at 4 °C) immediately after collection and transported the same day to the laboratory at the Research Centre on Health, Work and Environment (CISTA) at UNAN-León, where the clotted samples centrifuged at 3500 rpm within an hour of being received, and serum was transferred to five separate aliquots. The aliquots were stored at −20 °C.

Participants gave a spot urine sample (~50 cc) in sterile polypropylene containers, and aliquots were separated into vacuum tubes in the field immediately, placed in an icebox (4 °C) after collection and transported to the laboratory at the Research Center on Health, Work and Environment (CISTA) at UNAN-León), where aliquots were frozen −20 °C. Drinking water samples were collected in a bottle and stored at 4 °C.

Questionnaire

The questionnaire included socio-demographic information, work history, lifestyle, work conditions, liquid intake and current diseases that may be linked to CKD, specifically hypertension, diabetes, urinary tract and renal illness. The total time taken for the baseline interview was 1 to 1 ½ hours. The questionnaire was evaluated and tested for cognitive and linguistic suitability (See Additional file 1).

Interviewers obtained information on demographic characteristics (age, sex), socioeconomic status (education, income), water sources (location and type) and social security access (defined as access to a package of preventive, diagnostic and curative health services through the Nicaraguan government's Social Security System) [15]. Twenty-seven occupations stated by participants were regrouped using the International Standard Industrial Classification of all Economic Activities, Rev.4 (ISICv4) [16] into 10 economic activities which were further subdivided into occupational groups. Sugarcane workers were separated from other agricultural work groups as sugarcane workers have shown high prevalence rates of CKD [4]. Therefore occupations were grouped into only sugarcane, sugarcane with any other work (including other agricultural work), other agriculture work only, and work in neither agriculture nor sugarcane (see Table 2).

Using questionnaires (modified from those previous studies [1, 11, 17, 18]) exposure data were collected on heat stress, recurrent dehydration, physically demanding work, workplace conditions, pesticide exposure and potential exposure to heavy metals.

Finally, the participant's medical history was recorded. Urinary and renal illness was defined as a self-reported medically diagnosed urinary tract infection in the previous year, or a self-reported history of kidney disease or nephrolithiasis. Use of medications was ascertained by showing participants a visual catalogue of medication packages. Smoking status was classified according to whether participants used tobacco products daily, either currently or in the past. Questions on alcohol consumption and illicit drug use were also included.

Kidney function

For each participant, one aliquot of the serum sample was transported to the laboratory of Biochemistry at the Medical Faculty of UNAN-León, where serum creatinine (SCr) was measured with ChemWell® 2910 (Awareness Technology, EEUU) which is an automated assay based on the Jaffe compensated method [19–21]. SCr measurements were calibrated against an IDMS-traceable creatinine standard. The biochemistry laboratory at UNAN-León takes part in an international inter-laboratory quality control program, where measurements are compared to a laboratory standard (Serodos Plus Human Diagnostics, Wiesbaden, Germany) on a daily basis. In addition, for each batch of samples at least two duplicate serum samples were included for quality control purposes. Measured SCr values in the samples were at all times within the accepted limits of the method.

Kidney function was assessed using the estimated glomerular filtration rate according to the CKD-EPI formula by determining SCr during the baseline survey [22]. Future analyses, including serum Cystatin C determination, will be undertaken at the end of the follow-up period on stored aliquots.

Data analysis

The focus of this paper is on the study design and the findings of the baseline survey. Sociodemographic characteristics by sex were summarized using descriptive statistics. The continuous variables were examined using Kruskal-Wallis tests for non-normality and for categorical variables, the Pearson Chi-square test was used or Fisher's Exact Test when the chi-square was not applicable. Data were analysed with Stata software version 13.

Results

In nine communities in northwestern Nicaragua, 520 potential participants (286 men and 234 women) were identified in the population census. 16 males with CKD and 5 females with diabetes or hypertension were excluded from the study. Of the remaining population, all the males and 90 females, selected at random in order to have a 3:1 male:female ratio were invited to take part. Seven men and three women declined to participate after invitation. In total, 350 of the 360 invited participants attended baseline study visits (Fig. 2), with an average of 38 participants from each community (minimum of 26 and a maximum of 53).

Description of study population

Participants in all the communities had similar mean age (23 years), and median BMI (median 22.3 in men and 24.4 in women) (Table 1). The average number of members in a family was 4 persons. The majority of participants had relatively low schooling (mean 6.2 years for men and 7.1 years for women). The median systolic and diastolic blood pressures were 120/69 mmHg for men and 109/68 mmHg for women. All participants had a normal heart rate (median 69 for men and 77 for women. The mean household income per month in USD was just over $300. The eGFR of the participants ranged from 120 mL/min/1.73 m^2 to 137 mL/min/1.73 m^2, with a median value of 128 mL/min/1.73 m^2 for both men and women. Men had higher prevalence's of smoking tobacco and alcohol consumption. There were small differences in use NSAIDs between men and women. There were differences in urinary tract infections between sexes but not in use nephrotoxic antibiotics.

The associations between occupation and known/proposed risk factors for CKD are presented in Table 2. The prevalence of hypertension was low (2.9% of the participants). Nephrolithiasis was only reported among three women in the "Neither work in agriculture nor in sugarcane and only in sugarcane" but self-report of urinary tract infections were common in all groups (26% of the participants), except among male agricultural workers who had never worked in sugarcane (4%).

Baseline kidney function

Five percent of males had to be excluded from those identified in the initial population census because of pre-existing CKD. In addition, about one in 10 male participants had an estimated GFR less than 90 mL/min/1.73 m^2 (11%; Additional file 2: Table S1). Males with an eGFR <90 mL/min/1.73 m^2 were marginally older (24.6 years, compared with 23.0 years) and these participants had higher systolic and diastolic blood pressure. Participants who had an eGFR <90 mL/min/1.73 m^2, reported more frequent alcohol intake (86% vs 66%, $p = 0.03$). No other clinical measures (e.g., heart rate, BMI, education, income; Additional file 2: Table S1) were significantly associated with eGFR <90 mL/min/1.73 m^2. The prevalence of reduced kidney function was between 10 and 15% among men who had worked agriculture: 10% for sugarcane only (5/49), 15% for other agriculture only (20/172), and 12% for sugarcane with other work including other agriculture (4/27), whereas in contrast there were no cases among the men who had never worked in agriculture. The single woman with eGFR below 90 had never worked in agriculture (Table 2). Other associations between an eGFR <90 mL/min/1.73 m^2 and potential exposures are presented in the Additional file 2: Tables S1 and S2).

Discussion

Here we described the rationale, study design and baseline findings of a community based follow-up study in rural area of northwest Nicaragua. We have successfully

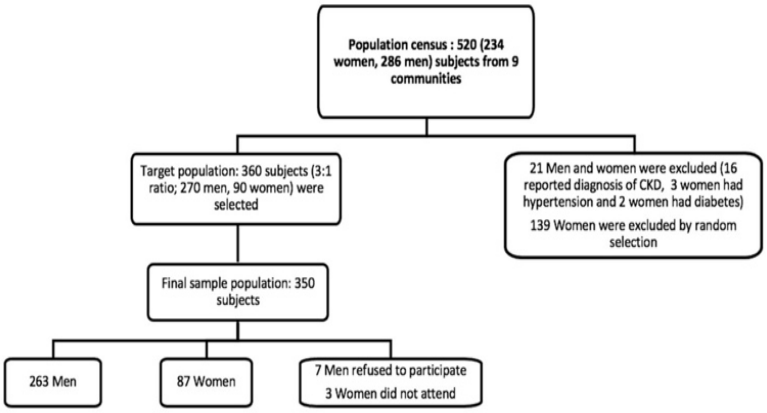

Fig. 2 Flow Chart outlining study population and recruitment. Overall participation from the final sample population was 97.2%

Table 1 Demographic characteristics of baseline population by sex

Variable	All participants	Sex	
		Men	Women
	n (350)	n (263)	n (87)
Age (yrs), mean ± SD	23 ± 3.7	23.3 ± 3.7	23.6 ± 3.5
Years of school, mean ± SD	6.4 ± 3.4	6.2 ± 3.4	7.1 ± 3.0
Household income per month (Córdobas), mean ± SD	7426 ± 5333	7744 ± 5531	6462 ± 4578
Number of family members, median (IQR)	4 (3–6)	4 (3–6)	4 (3–5)
Body mass index (BMI), median (IQR)	22.7 (21.0–29.9)	22.3 (20.8–24.1)	24.4 (21.8–30.0)
Systolic blood pressure (mmHg), median (IQR)	118 (109–124)	120 (111–126)	109 (103–119)
Diastolic blood pressure (mmHg), median (IQR)	68 (63–74)	69 (63–75)	68 (63–72)
Heart rate, median (IQR)	71 (65–78)	69 (64–76)	77 (71–83)
eGFR mL/min/1.73 m^2, median (IQR)	128 (121–137)	128 (120–137)	128 (123–136)
Ever smoked, # (%)	144 (41.1)	142 (53.0)	2 (2.3)
Ever drank alcohol, # (%)	194 (55.4)	181 (68.8)	13 (14.9)
NSAIDs[a], # (%)	296 (84.6)	217 (82.5)	79 (90.8)
Urinary tract infection, # (%)	91 (26.0)	56 (21.3)	35 (40.2)
Nephrotoxic antibiotics[b], # (%)	49 (14.0)	33 (12.5)	16 (18.4)

SD standard deviation, *eGFR* estimated glomerular filtration rate, *IQR* Interquartile range, *NSAIDs* nonsteroidal anti-inflammatory drugs
[a]diclofenac and ibuprofen; [b]gentamicin and amikacin

Table 2 Frequency of traditional risk factors by labour history in the baseline population

Labour history[a]	Sex (n)	Age	BMI	Years of Work life	eGFR mL/min/ 1.73 m^2	Prevalence of low GFR[b]	Prevalence of risk factors		
							Hypertension[c]	Nephrolithiasis	UTI
		Mean (SD)	Median (IQR)	Median (IQR)	Median (IQR)	n (%)	n (%)	n (%)	n (%)
Only sugarcane	Men (n: 49)	22.9 (3.6)	22.0 (20.4–24.2)	7.0 (3.6–10.2)	130 (121–139)	5 (10.2)	1 (2.0)	0 (0)	14 (28.6)
	Women (n: 14)	23.4 (3.9)	26.9 (21.8–32.2)	4.5 (3.0–9.7)	133 (123–138)	0 (0)	0 (0)	1 (7.1)	4 (28.6)
Sugarcane with any other work (including other agricultural work)	Men (n: 172)	23.9 (3.7)	22.6 (21.1–24.4)	9.1 (6.0–12.2)	127 (119–137)	20 (11.6)	2 (1.3)	1 (0.6)	37 (21.5)
	Women (n: 18)	24.3 (3.4)	28.1 (24.0–33.1)	10.1 (4.9–14.0)	126 (120–132)	0 (0)	0 (0)	0 (0)	8 (44.4)
Other agricultural work only	Men (n: 27)	21.2 (3.4)	21.8 (20.8–22.9)	6.0 (2.0–11.5)	130 (117–138)	4 (14.8)	1 (3.7)	0 (0)	1 (3.7)
	Women (n: 8)	22.1 (2.6)	23.7 (21.8–31.5)	8.0 (2.6–10.7)	128 (124–133)	0 (0)	1 (12.5)	0 (0)	2 (25.0)
Never worked in agriculture nor in sugarcane	Men (n: 15)	22.0 (4.3)	21.2 (19.4–22.5)	4.0 (2.0–11.0)	130 (123–145)	0 (0)	0 (0)	0 (0)	4 (26.7)
	Women (n: 47)	23.7 (3.6)	23.6 (21.4–28.1)	8.0 (4.0–12.5)	130 (126–137)	1 (2.1)	5 (10.6)	2 (4.3)	21 (44.7)
Total (350)		23.4 (3.7)	22.7 (21.0–24.9)	8.5 (4.5–12.0)	128 (121–137)	30 (8.6)	10 (2.9)	4 (1.1)	91 (26.0)

BMI Body mass index, *UTI* Urinary Tract Infection, *GFR* Glomerular Filtration Rate, *eGFR* Estimated Glomerular Filtration Rate, *IQR* Interquartile range
[a]Labour history categories grouped by current and previous occupation
[b]Low GFR: Low glomerular filtration rate was defined as eGFR <90 mL/min/1.73 m^2
[c]Hypertension: History of high blood pressure

partnered with a number of rural communities and achieved >90% participation rates at baseline.

The prevalence of early kidney dysfunction in this group of young apparently healthy adults provides further evidence of the devastating scale of impact of CKDu in agricultural workers in this region. Our baseline data presented here indicates that despite initially excluding participants with self-reported kidney disease, 11% of male participants had an eGFR <90 mL/min/1.73 m^2. The prevalence of lower eGFR amongst males in this area of northwest Nicaragua is consistent with the findings from previous studies [9, 23]. However, at this level of kidney function the eGFR calculated by the CKD Epi equation may over or underestimate the GFR by up to 30 mL/min, dependent on factors such as muscle mass and diet that vary between individuals. Therefore multiple measurements within individuals are needed to see whether these participants will go on to develop what would be clinically significant kidney dysfunction. The two-year follow-up design of our study will provide important data on the rate of decline of kidney function and will further explore which exposures are associated with within-person change in eGFR.

To perform community-based studies in the rural areas of Nicaragua, and other less economically developed countries, requires an awareness of a number of potential challenges. The study team has overcome bad road conditions to reach geographically isolated neighbourhoods (worsened by the rainy season) and frequent migration of the economically active population (due to lack of employment opportunities locally). Despite these problems, the response rate for the recruitment into the study was 97% of those initially identified as eligible participants.

This study demonstrates the importance of a locally-led, community-involved research team, which also has extensive experience conducting community based studies [1, 4, 11, 24]. Knowledge of the geographical area and experience regarding the social and cultural context has meant that many obstacles could be overcome.

Our study has several limitations. The study is only moderate sized due to the resources requirements for multiple follow-up visits. Furthermore, the delay before many of the analyses are performed may be frustrating for the participants. Finally, we are unable to quantify work exposures directly due to lack of access to workplace.

The main risk to the study going forward will be loss to follow-up due to internal and external migration. Rural communities have a tradition of working with seasonal crops and sugarcane workers often leave their communities at the end of each harvest season, to go abroad or to other regions within the country in search of temporary employment. With regular communication, community engagement and the maintenance of good relationships between researchers, community leaders and participants these problems should be minimised. A further challenge is to manage any potential negative consequences for participants taking part in the study. Sugarcane workers from nearby communities are reported to have lost their jobs as a result of participation in a prior cohort study [11]. In an attempt to mitigate against these types of consequences, the study team have written to local employers (including those in the sugarcane industry) explaining the content and extent of this study in order to reduce any concerns about workers' participation. In addition, the study team takes particular precautions to maintain participant's confidentiality during the study and beyond.

Conclusion

Community based follow-up studies have several advantages over cross-sectional studies in the community or research designs based in healthcare or occupational settings. These include generalizability, reduction in selection bias, better handling of reverse causation and recall bias, along with the ability to utilize an outcome measure (within-person change in eGFR) that allows the identification of those sustaining the most significant chronic kidney injury. The commitment and empowerment of the leaders of this community, and the extensive experience of fieldwork of the local researchers who are culturally embedded will be key to maintaining participant engagement and ensuring the success of this investigation.

Additional files

Additional file 1: Questionnaire. English transalation of the questionnaire used during the baseline study visit. (PDF 324 kb)

Additional file 2: Table S1. Demographic, anthropometrics characteristics, lifestyle and medical history among baseline participants of the cohort study. **Table S2.** Potential risk factors for heat stress among men and women. (PDF 88 kb)

Abbreviations

BMI: Body mass index; CISTA: Research center on health, work and environment; CKD: Chronic kidney disease; CKD-EPI: Chronic kidney disease epidemiology collaboration; CKDu: Chronic kidney disease of undetermined cause; eGFR: Estimated glomerular filtration rate; ESRD: End-stage renal disease; GFR: Glomerular filtration rate; IQR: Interquartile range; ISICv4: International standard industrial classification of all economic activities, Rev4; MeN: Mesoamerican nephropathy; NCDs: Non-communicable diseases; NSAIDs: Non-steroidal anti-inflammatory drugs; SCr: Serum creatinine; SD: Standard deviation; UK: United Kingdom; UNAN-León: National Autonomous University of Nicaragua, León; USD: United States Dollars; UTI: Urinary tract infection

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Availability of data and materials
All the data supporting this study are included within the manuscript and supplementary files. The dataset is available from the corresponding author.

Authors' contributions
This study was conceived by BC and NP and designed by MG, BC, NP, DN, CW, JG, JL, AA and LS. Data collection was performed by MG, AC, DF, JL, and BC. The analysis and interpretation of the results was done by MG, BC, DN, and NP. Draft was written by MG, BC, CW, DN, NP, AA. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
All participants signed a written informed consent to participate in the follow-up study, in accordance to the Declaration of Helsinki. The study was approved by the bioethical review board at the Medical Faculty of UNAN-León (Ref: FWA00004523/IRB00003342) and the research ethics committee of the London School of Hygiene and Tropical Medicine (Ref: 8643) in 2014.

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References

1. Torres C, Aragon A, Gonzalez M, Lopez I, Jakobsson K, Elinder CG, et al. Decreased kidney function of unknown cause in Nicaragua: a community-based survey. Am J Kidney Dis. 2010;55(3):485–96.
2. Ordunez P, Saenz C, Martinez R, Chapman E, Reveiz L, Becerra F. The epidemic of chronic kidney disease in Central America. Lancet Glob health. 2014;2(8):e440–1.
3. Correa-Rotter R, Wesseling C, Johnson RJ. CKD of unknown origin in Central America: the case for a Mesoamerican nephropathy. Am J Kidney Dis. 2014;63(3):506–20.
4. Raines N, Gonzalez M, Wyatt C, Kurzrok M, Pool C, Lemma T, et al. Risk factors for reduced glomerular filtration rate in a Nicaraguan community affected by Mesoamerican nephropathy. MEDICC Rev. 2014;16(2):16–22.
5. Wesseling C, Crowe J, Hogstedt C, Jakobsson K, Lucas R, Wegman DH. The epidemic of chronic kidney disease of unknown etiology in Mesoamerica: a call for interdisciplinary research and action. Am J Public Health. 2013;103(11):1927–30.
6. Wesseling C, Crowe J, Hogstedt C, Jakobsson K, Lucas R, Wegman DH, et al. Resolving the enigma of the mesoamerican nephropathy: a research workshop summary. Am J Kidney Dis. 2014;63(3):396–404.
7. Wegman D, Crowe J, Hogstedt C, Jakobsson K, Wesseling C, editors. Mesoamerican nephropathy: report from the second international research workshop on MeN. Heredia, C.R: SALTRA/IRET-UNA; 2016. Contract No.: ISBN 978-9968-924-33-7.
8. Caplin B, González-Quiroz M, Pearce N. Gaining insights into the evolution of CKDnt from community-based follow up studies. In: SALTRA, editor. Second International workshop on mesoamerican nephropathy; San José. Costa Rica: SALTRA; 2015.
9. Sanoff SL, Callejas L, Alonso CD, Hu Y, Colindres RE, Chin H, et al. Positive association of renal insufficiency with agriculture employment and unregulated alcohol consumption in Nicaragua. Ren Fail. 2010;32(7):766–77.
10. Laws RL, Brooks DR, Amador JJ, Weiner DE, Kaufman JS, Ramirez-Rubio O, et al. Changes in kidney function among Nicaraguan sugarcane workers. Int J Occup Environ Health. 2015;21(3):241–50.
11. Wesseling C, Aragon A, Gonzalez M, Weiss I, Glaser J, Bobadilla NA, et al. Kidney function in sugarcane cutters in Nicaragua - A longitudinal study of workers at risk of Mesoamerican nephropathy. Environ Res. 2016;147:125–32.
12. Padala S, Tighiouart H, Inker LA, Contreras G, Beck GJ, Lewis J, et al. Accuracy of a GFR estimating equation over time in people with a wide range of kidney function. Am J Kidney Dis. 2012;60(2):217–24.
13. de Boer IH, Sun W, Cleary PA, Lachin JM, Molitch ME, Zinman B, et al. Longitudinal changes in estimated and measured GFR in type 1 diabetes. J Am Soc Nephrol. 2014;25(4):810–8.
14. Boucquemont J, Heinze G, Jager KJ, Oberbauer R, Leffondre K. Regression methods for investigating risk factors of chronic kidney disease outcomes: the state of the art. BMC Nephrol. 2014;15:45.
15. Thornton RL, Hatt LE, Field EM, Islam M, Diaz FS, Gonzalez MA. Social security health insurance for the informal sector in Nicaragua: a randomized evaluation. Health Econ. 2010;19(Suppl):181–206.
16. International Standard Industrial Classification of all Economic Activities, Rev. 4. Neork, USA: United Nations; 2016 [cited 2016 02/08]. Available from: http://unstats.un.org/unsd/cr/registry/regcst.asp?Cl=27.
17. Garcia-Trabanino R, Jarquin E, Wesseling C, Johnson RJ, Gonzalez-Quiroz M, Weiss I, et al. Heat stress, dehydration, and kidney function in sugarcane cutters in El Salvador - A cross-shift study of workers at risk of Mesoamerican nephropathy. Environ Res. 2015;142:746–55.
18. Wesseling C, Aragon A, Gonzalez M, Weiss I, Glaser J, Rivard C, et al. Heat stress, hydration and uric acid: a cross-sectional study in workers of three occupations in a hotspot of mesoamerican nephropathy in Nicaragua. BMJ Open. 2016;6(12):1–12.
19. Wyss M, Kaddurah-Daouk R. Creatine and creatinine metabolism. Physiol Rev. 2000;80(3):1107–213.
20. Roche D. Creatinine Jaffe Gen. 2, Compensated Method for serum and Plasma. Mannheim: Roche Diagnostics; 2006.
21. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis. 2002;39(2 Suppl 1):S1–266.
22. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med. 2012;367(1):20–9.
23. Lebov JF, Valladares E, Pena R, Pena EM, Sanoff SL, Cisneros EC, et al. A population-based study of prevalence and risk factors of chronic kidney disease in Leon, Nicaragua. Can J Kidney Health Dis. 2015;2:6.
24. Laux TS, Bert PJ, Barreto Ruiz GM, Gonzalez M, Unruh M, Aragon A, et al. Nicaragua revisited: evidence of lower prevalence of chronic kidney disease in a high-altitude, coffee-growing village. J Nephrol. 2012;25(4):533–40.

3.5 Supplementary material

Supplementary table 1: Demographic and anthropometric characteristics, lifestyle factors and medical history among baseline participants in the cohort study

Variables	Men (n: 263)			Women (n: 87)		
	eGFR (<90 mL/min/1.73 m^2)*			eGFR (<90 mL/min/1.73 m^2)*		
	Yes (n: 29, 11.0%)	No (n: 234, 90.0%)	P-value	Yes (n: 1, 1.1%)	No (n: 86, 98.9%)	P-value
Age (yrs), mean ± SD**	24.7± 2.9	23.1 ± 3.8	0.022	28.0 ± 0.0	23.6 ± 3.5	0.202
Systolic blood pressure, mean ± SD**	125.1 ± 10.3	118.1 ± 9.1	0.002	113.0 ± 0.0	111.1 ± 11.5	0.765
Diastolic blood pressure, mean ± SD**	75.2 ± 7.1	68.8 ± 7.3	0.001	86.0 ± 0.0	68.8 ± 8.7	0.135
Heart rate, mean ± SD**	72.6 ± 9.8	70.3 ± 8.4	0.256	86.0 ± 0.0	77.3 ± 8.3	0.209
BMI, mean ± SD**	24.0 ± 3.7	22.8 ± 3.5	0.092	21.4 ± 0.0	26.3 ± 6.1	0.359
BMI > 25 kg/m^2***	6 (20.7%)	41 (17.5%)	0.674	0 (0%)	40 (46.5%)	---
Education (yrs), mean ± SD**	6.2 ± 4.1	6.2 ± 3.4	0.719	6.0 ± 0.0	7.2 ± 3.1	0.765
Household income per person in family (córdobas; 26.72 = 1 US$), mean ± SD **	7340 ± 4823	7794 ± 5620	0.802	4000.0 ± 0	6491 ± 4597	0.536
Total number of family members who depend on the household income**	4.3 ± 1.6	4.9 ± 2.4	0.461	3.0 ± 0.0	4.5 ± 2.2	0.324
Age of first job, paid or unpaid, mean ± SD**	14.3 ± 3.2	14.6 ± 3.2	0.756	18.0 ± 0.0	15.3 ± 3.8	0.389
Ever smoked***	20 (69.0%)	122 (52.1%)	0.086	0 (0%)	2 (2.3%)	---
Ever drank alcohol***	25 (86.2%)	156 (66.6%)	0.032	1 (100.0%)	12 (14.0%)	---
NSAIDs***	22 (75.9%)	195 (83.3%)	0.318	0 (0%)	79 (91.9%)	---
Nephrotoxic antibiotics***	6 (20.7%)	27 (11.5%)	0.161	0 (0%)	16 (18.6%)	---
Hypertension***	2 (6.9%)	2 (0.9%)	0.061	0 (0%)	6 (7.0%)	---
History diabetes***	0 (0%)	0 (0%)	---	0 (0%)	0 (0%)	---
History kidney stones***	0 (0%)	1 (0.4%)	---	0 (0%)	3 (3.4%)	---
History urinary tract infections (UTI)***	8 (27.5%)	48 (20.5%)	0.470	0 (0%)	35 (40.6%)	---

** eGFR was defined as eCKD-Epi GFR <90 mL/min/1.73 m^2. ** Kruskal-Wallis test for non-normally distributed continuous variables. *** Chi-square test for categorical variables or Fisher's exact test at expected frequencies <5.*

Abbreviations: BMI: body mass index, eGFR: estimated glomerular filtration rate, NSAIDs: non-steroidal anti-inflammatory drugs.

Supplementary table 2: Potential risk factors for heat stress among men and women

Variables	Men (n: 263) eGFR (<90 mL/min/1.73 m²)*			Women (n: 87) eGFR (<90 mL/min/1.73 m²)*	
	Yes (n: 29, 11.0%)	No (n: 234, 90.0%)	P-value	Yes (n: 1, 1.1%)	No (n: 86, 98.9%)
Years of working life, mean ± SD**	10.2 ± 3.9	8.4 ± 4.7	0.032	10.0	8.2 ± 4.9
Work hours per day, mean ± SD**	7.1 ± 1.9	7.2 ± 2.4	0.890	10.0	8.0 ± 2.0
Total duration breaks per day (minutes), mean ± SD**	29.8 ± 27.1	22.7 ± 20.1	0.378	60.0	45.0 ± 45.2
Number of breaks per day, mean ± SD	2.4 ± 1.5	2.5 ± 1.6	0.651	3.0	2.3 ± 1.0
Very rapid work pace (%)***	5 (17.2%)	91 (38.8%)	0.022	No	27 (31.3%)
Hot environment in current job (%)***	5 (17.2%)	47 (20.1%)	0.717	No	3 (3.5%)
Lifting weights > 50 pounds (%)***	6 (20.6%)	54 (23.0%)	0.773	No	5 (5.8%)
Physical effort in the last week	16 (55.1%)	133 (56.8%)	0.864	No	44 (51.1%)
Ever fainted due to work (%)***	2 (6.8%)	8 (3.4%)	0.304	No	5 (5.8%)
Weight loss in current job (self-reported)***	8 (27.5%)	85 (36.3%)	0.353	No	30 (34.8%)

eGFR was defined as eGFR <90 mL/min/1.73 m²; ** Kruskal-Wallis test for non-normally distributed continuous variables;* Chi-square test for categorical variables or Fisher's exact test at expected cell frequencies <5.*

Chapter 4. Community-based longitudinal study- An unparalleled decline in kidney function among young adults

4.1 Introduction to paper III

This paper has been published in Journal of the American Society of Nephrology (JASN) and presents the natural history of and factors associated with the loss of kidney function in a high-risk rural population in northwest Nicaragua. This study used prospective longitudinal data to identify the subgroups of kidney function trajectories among young adults aged 18-30 years. Details of the cohort protocol were described in the rationale and baseline findings were published paper II and described in chapter 3.

A key aim was to describe the natural history of CKDu over time. Cross-sectional studies have described that CKDu undergoes a fast progression to end stage renal disease, but no reports have previously examined the rate of decline in kidney function. This result prompted us to design a study to understand the prognosis and how disabling or lethal this disease is among a disadvantage population.

A further aim was to identify risk factors associated with a future rapid decline in kidney function. The associations of a number of proposed risk factors (sociodemographic data, occupational exposure, heat-stress, self-medication, habits, dehydration symptoms, etc.) with the rapid decline were examined and a sensitivity analysis was conducted with the exposure data that were collected

solely after the harvest season (six months after the first visit for most participants).

Initially, the longitudinal data were analysed using a multilevel model, however, there was evidence of poor model fit and violation of the statistical assumptions required for such models. Residuals were not randomly distributed around individual slopes and did not follow a normal distribution. Based on this issue, we decided to use a growth mixture modelling approach because this allow us to identify classes of individual with similar trajectory of decline over time or with similar clinical outcome. This approach was the most appropriate method for fully capturing information about interindividual variations, heterogeneity of different groups within a population and the statistical assumptions underlying growth mixture modelling were met. However, this analysis raises a limitation when the number of affected people in each subgroup of decline in kidney function is too small leading to reduced power to detect association.

Tables and figures that describe the sociodemographic characteristics and sensitivity analysis were included in this paper as supplementary materials. All of these tables and figures are included in this chapter as section 4.5.

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Principal Supervisor	Dorothea Nitsch
Thesis Title	Occupational kidney disease among young populations in northwest Nicaragua

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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I designed the study in collaboration with Dorothea Nitsch, Catharina Wesseling, Jason Glasser, Jennifer LeBlond, Aurora Aragón, Liam Smeeth, Neil Pearce and Ben Caplin. I also led the fieldwork and data collection along with the fieldwork team and Armando Camacho, Dorien Faber, Jennifer LeBlond and Ben Caplin. I entered and analysed the data and interpreted the results under the supervision of Richard Silverwood, Dorothea Nitsch, Niel Pearce and Ben Caplin. The biological samples were analysed by Evangelia-Theano Smpokou, Brenda de la Rosa Garcia, Amin Oomantia, and Michael Hill. I drafted the manuscript according to the advice of by Richard Silverwood, Dorothea Nitsch, Neil Pearce and Ben Caplin. All co-authors read and approved the final manuscript that has submitted to JASN. My co-authors and I have edited the draft and addressed the reviewer's comments during peer review process.

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4.4 Research paper cover sheet: *Decline in kidney function among apparently healthy young adults at risk of Mesoamerican nephropathy*

Decline in Kidney Function among Apparently Healthy Young Adults at Risk of Mesoamerican Nephropathy

Marvin Gonzalez-Quiroz,[1,2,3] Evangelia-Theano Smpokou,[3] Richard J. Silverwood,[4] Armando Camacho,[1] Dorien Faber,[5] Brenda La Rosa Garcia,[3] Amin Oomatia,[3] Michael Hill,[6] Jason Glaser,[7] Jennifer Le Blond,[8] Catharina Wesseling,[9] Aurora Aragon,[1] Liam Smeeth,[2] Neil Pearce,[2,4] Dorothea Nitsch,[2] and Ben Caplin[3]

Due to the number of contributing authors, the affiliations are listed at the end of this article.

ABSTRACT

Background Epidemic levels of CKD of undetermined cause, termed Mesoamerican nephropathy in Central America, have been found in low- and middle-income countries. We investigated the natural history of, and factors associated with, loss of kidney function in a population at high risk for this disease.

Methods We conducted a 2-year prospective, longitudinal study with follow-up every 6 months in nine rural communities in northwestern Nicaragua and included all men (*n*=263) and a random sample of women (*n*=87) ages 18–30 years old without self-reported CKD, diabetes, or hypertension. We used growth mixture modeling to identify subgroups of eGFR trajectory and weighted multinomial logistic regression to examine associations with proposed risk factors.

Results Among men, we identified three subpopulations of eGFR trajectory (mean baseline eGFR; mean eGFR change over follow-up): 81% remained stable (116 ml/min per 1.73 m^2; −0.6 ml/min per 1.73 m^2 per year), 9.5% experienced rapid decline despite normal baseline function (112 ml/min per 1.73 m^2; −18.2 ml/min per 1.73 m^2 per year), and 9.5% had baseline dysfunction (58 ml/min per 1.73 m^2; −3.8 ml/min per 1.73 m^2 per year). Among women: 96.6% remained stable (121 ml/min per 1.73 m^2; −0.6 ml/min per 1.73 m^2 per year), and 3.4% experienced rapid decline (132 ml/min per 1.73 m^2; −14.6 ml/min per 1.73 m^2 per year; *n*=3 women). Among men, outdoor and agricultural work and lack of shade availability during work breaks, reported at baseline, were associated with rapid decline.

Conclusions Although Mesoamerican nephropathy is associated with agricultural work, other factors may also contribute to this disease.

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CKD of undetermined cause (CKDu), also termed Mesoamerican nephropathy (MeN) in Central America, has led to the deaths of tens of thousands of young adults in rural Nicaragua and El Salvador.[1,2] Cross-sectional studies have shown low (<60 ml/min per 1.73 m^2) eGFR at a prevalence between 2% and 50% among the population of lowland agricultural communities in the region.[3–6] Forms of CKDu occur in other tropical climates, with reports of high prevalence in Sri Lanka[7,8] (where similar but not identical histopathologic findings have been reported[9]), India,[10] and Egypt,[11] although whether this represents the same disease entity remains unclear.

Men from communities affected by MeN predominantly work in agriculture, primarily sugar production from cane. Agricultural activity in this industry is concentrated in the dry season, which in

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Nicaragua, occurs between November and May. Although a leading hypothesis in Mesoamerica is that the disease relates to heat stress, a number of other causes, including agrichemicals, infection, and heavy metals, have been proposed.[1,12–14]

Empirical evidence for causes of CKDu has to date been limited to identification of factors associated with either reduced eGFR in cross-sectional studies[3,15,16] or loss of eGFR across the harvest season in two workplace-based follow-up studies.[17,18] Given the potential for reverse causation (*i.e.*, reduced eGFR resulting in changes in exposure) and misclassification of exposures and/or outcome in the cross-sectional designs along with the nongeneralizability and the substantial loss to follow-up that occurred in the longitudinal workplace studies, evidence on risk factors for and evolution of CKDu is extremely limited.[19]

Our aim was to investigate the natural history of disease, specifically early loss of kidney function, along with risk factors and urinary markers (albumin-to-creatinine ratio [ACR] and neutrophil gelatinase–associated lipocalin [NGAL]) associated with decline in eGFR. Therefore, we conducted a community-based longitudinal study of an initially apparently healthy young rural population in northwest Nicaragua.

Significance Statement

CKD of undetermined cause is the leading cause of death among working-age adults in a number of Central American countries. This is the first community-based, longitudinal study undertaken in the at-risk population. The results show striking evolution of disease with a substantial proportion of initially apparently healthy men and a small number of women experiencing rapid loss of kidney function over the 2-year follow-up. Although a number of occupational risk factors were identified, the range of study participants who sustained loss of eGFR suggests that other factors also play a role. These findings describe a highly prevalent, uniquely aggressive kidney disease with no clear cause. Gaining insight into the etiology should be a global health research priority.

METHODS

Cohort

Both local and United Kingdom–based institutional review boards approved the study, and participants provided written informed consent. The rationale and description of the study design have been published elsewhere.[20] Briefly, this was a 2-year longitudinal, community-based study following 350 participants ages 18–30 years old in the Leon and Chinandega regions of Nicaragua (Figure 1). After engagement work, we performed a census of all adults ages 18–30 years old in nine rural communities. Because we were specifically interested in associations with early kidney injury in MeN, all potential participants with a self-reported diagnosis of CKD, diabetes, or hypertension were excluded. All remaining men (because men have been reported to suffer more CKDu) and women selected at random (in numbers leading to a men-to-women ratio of 3:1) were invited to take part. Participants were predominantly recruited in November 2014, with an additional 7% recruited in May 2015, because recruitment targets had not been met in November.

Procedures

Questionnaire data, clinical measurements, and biosamples were collected at baseline and then every 6 months until November 2016. Participants were asked to respond to questions on demography, occupational history and current job, lifestyle factors, and symptoms. Urinary tract infection was recorded where participants reported a clinical diagnosis (which is common in this part of Nicaragua), typically without urinalysis or microbiologic confirmation. Body weight was measured with minimal clothes using electronic scales (Seca, Birmingham, United Kingdom), and height was measured using a portable stadiometer (Seca). BP and heart rate were measured in a sitting position using a calibrated digital sphygmomanometer (Omron, Kyoto, Japan) after 5 minutes of quiet seated rest. A mean of three measurements was recorded. Participants were asked to attend fasted first thing in the morning (before work) in an attempt to reduce within- and between-person variation in serum creatinine.

Biochemical Methods

Serum creatinine and cystatin C were both measured in a single batch using quality control referenced to international standards (for creatinine, isotope dilution mass spectrometry–quantified National Institute of Standards and Technology Standard Reference Material 967). eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula combining creatinine and cystatin C.[21] ACR along with semiquantitative protein and specific gravity by test stick were performed in baseline urine samples thawed for the first time. In addition, 55 samples (thawed for a second time) selected using a nested patient-control approach were analyzed for NGAL.

Statistical Methods

The collection and categorization of exposure variables are described in Supplemental Material. Because eGFR trajectories clustered in discrete subgroups (Supplemental Figures 1 and 2) and differently between sexes, we used growth mixture modeling (GMM) separately in men and women to empirically derive latent classes of eGFR trajectory.[22] The GMM is a longitudinal finite mixture model that allows identification of unobserved latent classes of individuals following similar progression of the outcome over time without imposing *a priori* constraints on the levels of eGFR or rates of eGFR change (or the proportion of participants experiencing any class of change). Each individual's probability of belonging to a particular latent class is derived entirely from the observed eGFR measurements, with individual departures from the mean trajectory within each class represented by random effects. We primarily used the Bayesian Information Criterion to determine the optimal number of classes as suggested in this setting.[23] The GMM was estimated by

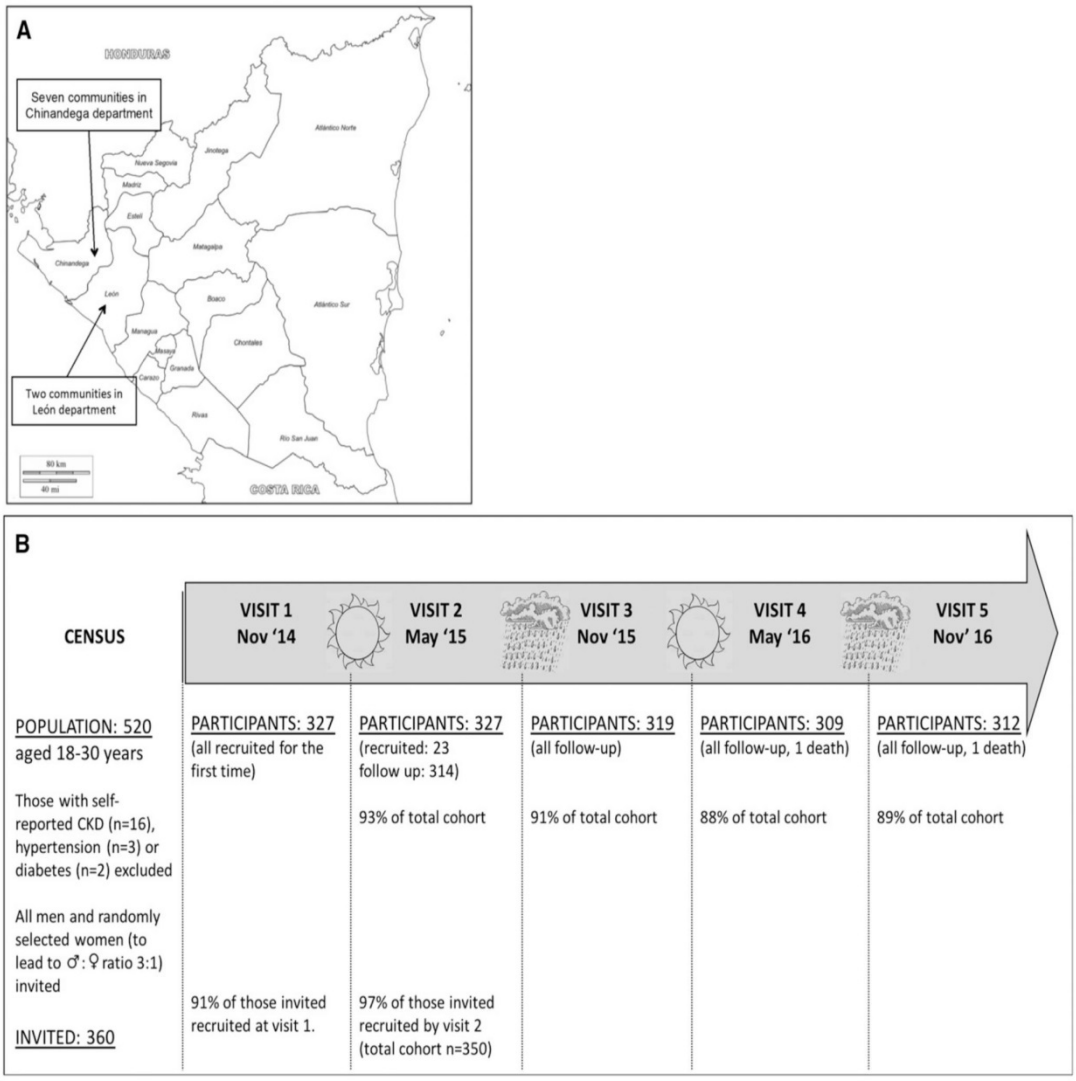

Figure 1. Study participants were recruited from nine communities in Northwest Nicaragua and study retention rates were high. (A) Location of the nine study communities in Nicaragua. (B) Cartoon showing the study timeline along with population, recruitment, and follow-up numbers. Two participants died from ESRD.

maximum likelihood using an expectation maximization algorithm, with 95% confidence intervals (95% CIs) for the mean rate of eGFR decline derived using conventional SEM.

Each individual was assigned a probability of each class (eGFR trajectory) and then for the purposes of the descriptive figure, tables, and urinary findings, allocated to the highest probability group.

To test whether proposed causal exposures (alcohol or nonsteroidal anti-inflammatory drug use, occupational factors, heat stress, agrochemical exposure, fever, dysuria, or water quantity/quality/source in men only) were associated with rapidly declining eGFR trajectory, we conducted age- and educational level–adjusted analyses using probability-weighted logistic regression (with weighting according to the participant's probability of each eGFR trajectory as per the GMM), examining exposures individually using stable with preserved eGFR trajectory as a reference. Associations where the 95% CI of the odds ratio (OR) did not include unity were interpreted as significant. We also performed a sensitivity analysis using exposures assessed at visit 2 (only in those men recruited at visit 1) and rapid decline given the seasonal variation in occupational exposures. Those with baseline dysfunction were not the primary focus of this study, but another analysis additionally exploring associations between risk factors and this eGFR trajectory was also performed using probability-weighted logistic regression (Supplemental Material).

Differences in urinary markers in each eGFR trajectory group (defined on the basis of the highest probability as above) were investigated either in the whole population for ACR or using a nested patient-control approach in the case of NGAL.

Table 1. Selected demographic, lifestyle, and occupational characteristics of the study cohort

Characteristic	Overall, *n*=350	Men, *n*=263	Women, *n*=87
Personal and lifestyle factors			
Age, yr, mean (SD)	23.9 (3.7)	23.7 (3.8)	24.2 (3.6)
Educational level, *n* (%)			
Illiteracy	18 (5.1)	18 (6.8)	0 (0)
Primary school	176 (50.3)	133 (50.6)	43 (49.4)
Secondary school	138 (39.5)	100 (38.0)	38 (43.7)
Higher education	18 (5.1)	12 (4.6)	6 (6.9)
Body mass index, median (IQR)	22.7 (21.0–25.0)	22.4 (20.8–24.1)	24.5 (21.9–30.0)
Systolic BP, mm Hg, median (IQR)	117 (109–124)	119 (111–125)	109 (103–119)
Diastolic BP, mm Hg, median (IQR)	68 (63–73)	68 (63–74)	68 (63–72)
Household income, Córdobas per 1 mo, median (IQR)	6000 (4000–9200)	6000 (4000–10,000)	5120 (3380–8144)
Family history of CKD, *n* (%)			
Yes	165 (47.1)	126 (47.9)	39 (44.8)
No	185 (52.9)	137 (52.1)	48 (55.2)
Annual alcohol consumption, g, median (IQR)	0·0 (0–849)	82.9 (0–1350)	0.0 (0–0)
Smoking pack-year, median (IQR)	0·0 (0–0)	0·0 (0–1)	0.0 (0–0)
NSAID use ever, *n* (%)			
Never	58 (16.6)	49 (18.6)	9 (10.3)
Occasionally	251 (71.7)	185 (70.3)	66 (75.9)
Regularly	31 (8.9)	23 (8.8)	8 (9.2)
Daily	10 (2.8)	6 (2.3)	4 (4.6)
Water sources, *n* (%)			
Piped water	186 (53.1)	139 (52.9)	47 (54.0)
Dug well	126 (36.0)	98 (37.2)	28 (32.2)
Drilled well	38 (10.9)	26 (9.9)	12 (13.8)
Water hardness, *n* (%)			
Soft	0 (0.0)	0 (0.0)	0 (0.0)
Moderately hard	97 (27.7)	67 (25.4)	30 (34.5)
Hard	160 (45.7)	123 (46.8)	37 (42.5)
Very hard	93 (26.6)	73 (27.8)	20 (23.0)
Total liquid in last 24 h, median (IQR)	5.0 (3.7–6.3)	5.6 (4.2–6.7)	3.6 (2.5–4.5)
Occupational factors			
Current occupation, *n* (%)			
Sugarcane	55 (15.7)	45 (17.1)	10 (11.5)
Banana work	14 (4.0)	13 (4.9)	1 (1.1)
Other agricultural work	115 (32.9)	109 (41.5)	6 (6.9)
Commerce	14 (4.0)	5 (1.9)	9 (10.3)
Construction	10 (2.9)	10 (3.8)	0 (0)
Fishing	7 (2.0)	7 (2.7)	0 (0)
Homeworker	54 (15.4)	0 (0)	54 (62.1)
Student	6 (1.7)	4 (1.5)	2 (2.3)
Unemployed	51 (14.6)	49 (18.6)	2 (2.3)
Other occupations[a]	24 (6.8)	21 (8.0)	3 (3.5)
Main sugarcane role (if ever worked in sugarcane), *n* (%)			
Cane cutter	81 (23.2)	81 (30.8)	0 (0)
Seed cutter	56 (16.3)	56 (21.3)	0 (0)
Seeder	67 (19.2)	47 (17.9)	21 (24.1)
Cane cleaner	26 (7.4)	17 (6.5)	9 (10.4)
Pesticide applicator	4 (1.1)	4 (1.5)	0 (0)
Cane irrigator	8 (2.3)	8 (3.0)	0 (0)
Driver	4 (1.1)	4 (1.5)	0 (0)
Never worked in sugarcane	103(29.4)	46 (17.5)	57 (65.5)

Table 1. Continued

Characteristic	Overall, *n*=350	Men, *n*=263	Women, *n*=87
Current or previous banana work, *n* (%)			
Yes	56 (16.0)	47 (17.9)	9 (10.3)
No	294 (84.0)	216 (82.1)	78 (89.7)
Years in sugarcane, mean (SD)	2.2 (2.8)	2.8 (2.8)	0.67 (1.7)
Years in agricultural, mean (SD)	3.6 (4.4)	4.3 (4.5)	1.2 (3.3)
Work carried out,[b] *n* (%)			
Indoors	136(38.9)	69 (26.2)	67 (77.0)
Outdoors	214 (61.1)	194 (73.8)	20 (23.0)
Work in a hot environment,[b] *n* (%)			
Irregularly	137 (39.2)	92 (35.0)	45 (51.7)
Regularly	74 (21.1)	57 (21.7)	17 (19.5)
Frequently	139 (39.7)	114 (43.3)	25 (28.8)
Always	0 (0)	0 (0)	0 (0)
Shade availability,[b] *n* (%)			
Yes	254 (72.6)	190 (72.2)	64 (73.6)
No	96 (27.4)	73 (27.8)	23 (26.4)
Duration of breaks, min,[b] median (IQR)	20 (10–30)	15.0 (10–30)	30.0 (20–60)
Physical effort at work,[c] *n* (%)			
Did not work	15 (4.3)	14 (5.3)	1 (1.2)
Slight	142 (40.6)	100 (38.0)	42 (48.3)
Moderate	155 (44.2)	119 (45.3)	36 (41.4)
Hard	38 (10.9)	30 (11.4)	8 (9.2)
Glyphosate use,[b,d] *n* (%)			
Yes	77 (22.0)	77 (29.3)	0 (0)
No	273 (78.0)	186 (70.7)	87 (100.0)
Paraquat use,[b,d] *n* (%)			
Yes	44 (12.6)	44 (16.7)	0 (0)
No	306 (87.4)	219 (83.3)	87 (100.0)
Cypermethrin use,[b,d] *n* (%)			
Yes	75 (21.4)	73 (27.7)	2 (2.3)
No	275 (78.6)	190 (72.2)	85 (97.7)
Methomyl use,[b,d] *n* (%)			
Yes	12 (3.4)	12 (4.6)	0 (0)
No	338 (96.6)	251 (94.4)	87 (100.0)
Clinical history/symptoms			
Heat/dehydration symptoms,[b] *n* (%)			
Yes	240 (68·6)	175 (66·5)	65 (74·7)
No	110 (31·4)	88 (33·5)	22 (25·3)
UTI in the previous year, *n* (%)			
Yes	91 (26.0)	56 (21.3)	35 (40.2)
No	259 (74.0)	207 (78.7)	52 (59.8)
Weight loss,[b] *n* (%)			
Yes	63 (18.0)	55 (20.9)	8 (9.2)
No	287 (82.0)	208 (79.1)	79 (90.8)
Dysuria[b]			
Yes	94 (26.9)	72 (27.4)	22 (25.3)
No	256 (73.1)	191 (72.6)	65 (74.7)
Fever[b]			
Yes	36 (10.3)	32 (12.2)	4 (4.6)
No	314 (89.7)	231 (87.8)	83 (95.4)
Study visits and outcome			
Initial serum creatinine, mg/dl, median (IQR)	0.81 (0.70–0.90)	0.84 (0.77–0.94)	0.63 (0.57–0.68)
Final serum creatinine, mg/dl, median (IQR)	0.81 (0.70–0.90)	0.91 (0.80–1.03)	0.64 (0.57–0.72)

Table 1. Continued

Characteristic	Overall, *n*=350	Men, *n*=263	Women, *n*=87
Initial cystatin C, mg/L, median (IQR)	0.82 (0.74–0.92)	0.85 (0.77–0.95)	0.72 (0.67–0.80)
Final cystatin C, mg/L, median (IQR)	0.84 (0.76–0.94)	0.88 (0.80–1.01)	0.72 (0.67–0.80)
Initial eGFR, ml/min per 1.73 m^2, median (IQR)	118.3 (106.6–125.4)	116.2 (102.4–124.6)	122.0 (116.3–127.2)
Final eGFR, ml/min per 1.73 m^2, median (IQR)	113.1 (99.4–122.3)	110.4 (92.5–120.1)	120.2 (110.6–126.6)

eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation on the basis of creatinine and cystatin c. Questionnaire data before recoding are presented in Supplemental Table 1. IQR, interquartile range. NSAID, nonsteroidal anti-inflammatory drug; UTI, diagnosed with a urinary tract infection typically without microbiologic or dipstick confirmation.[a]Other occupations include teacher, painter, shoemaker, security, manufacturing operator, and barber.
[b]Over the last 6 months.
[c]Over the last week.
[d]Data were collected at the second visit.

Differences between groups were explored using ANOVA with the Dunnett post hoc test. Positive and negative predictive values were calculated for urinary NGAL for the rapid decline versus stable group.

RESULTS

Cohort and Follow-Up

Five hundred twenty adults ages 18–30 years old were identified in the study communities. After exclusion of 4% of the potential participants because of self-reported CKD, diabetes, or hypertension, 350 participants (of the 360 invited after random selection of eligible women; 97%) were included in the study.[20] Overall, participants attended a total of 1581 study visits over the 2-year follow-up (92% of planned visits). Two participants died from ESRD during the study period. The cohort is described in Figure 1 and Table 1.

The median eGFR in men was 116.2 ml/min per 1.73 m^2 (interquartile range [IQR], 102.4–124.6) at baseline, and 110.4 ml/min per 1.73 m^2 (IQR, 92.5–120.5) at the end of follow-up. The corresponding figures for women were 122.0 ml/min per 1.73 m^2 (IQR, 116.3–127.2) at baseline and 120.2 ml/min per 1.73 m^2 (IQR, 110.6–126.6) at the end of follow-up. The eGFR varied by season (Figure 2), with a median of 116.0 ml/min per 1.73 m^2 (IQR, 102.7–123.8) at the end of the rainy season (November; *i.e.*, before sugarcane harvest, all years combined) compared with 113.4 ml/min per 1.73 m^2 (IQR, 100.8–122.4) at the end of the dry season (May; *i.e.*, after sugarcane harvest, all years combined). This effect was greatest in those participants with lower eGFRs, but it was also present in those with stable kidney function (Supplemental Table 2).

eGFR Trajectory Groups

Using GMM, we identified three different subgroups in men and two subgroups in women on the basis of the model intercept (baseline eGFR) and slope (change in eGFR over time). Among men (Figure 3A), the majority (81%) of men had preserved and stable eGFR; however, 9.5% (*n*=25) had baseline kidney dysfunction (eGFR of approximately 60 ml/min at recruitment), and another 9.5% experienced rapid decline in eGFR (with a mean loss of 18 ml/min per 1.72 m^2 per year) despite preserved eGFR at baseline. Almost all of the women (Figure 3B) had preserved and stable eGFR, but 3.4% (*n*=3) also experienced rapid decline (with a mean loss of 14 ml/min per 1.72 m^2 per year). No differences were seen between communities in the proportions of participants in these subgroups.

Baseline sociodemographics, occupational history, occupational exposures, lifestyle factors, and symptoms stratified by the assigned kidney trajectory groups are presented in Supplemental Tables 2 and 3. The frequencies of indoor work and availability of shade were both lower in the rapidly declining subgroup. Of the three women who fell into the rapid decline group, one had worked in (nonsugarcane) agriculture, and two worked exclusively at home.

Adjusted Associations with Rapid Decline Trajectory

Baseline age– and educational level–adjusted probability-weighted associations with the rapid decline in eGFR

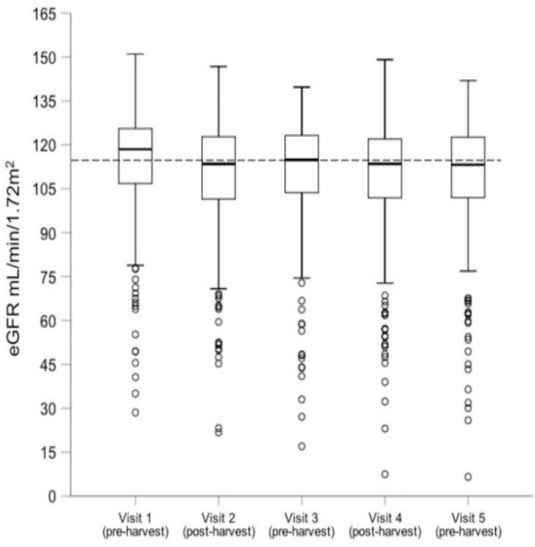

Figure 2. Median eGFR was lower postharvest than preharvest. Box and whisker plot of eGFR across the study population. The dashed line represents the median of all eGFR values in the population across the study.

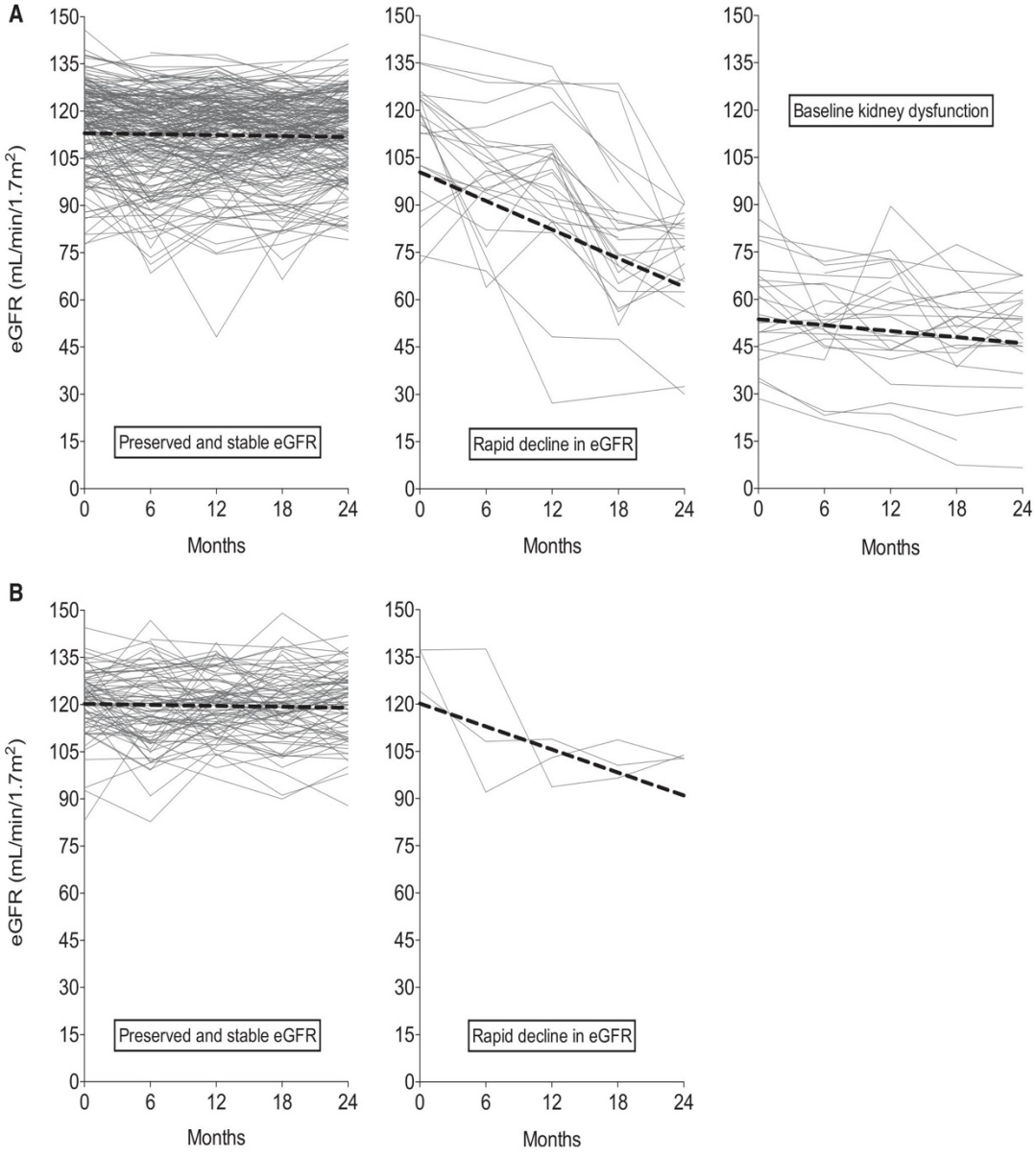

Figure 3. A substantial proportion of men and a small number of women experience rapid decline in eGFR. Individual eGFR values over time stratified by trajectory subgroup. (A) Three subgroups were identified in 263 men, and (B) two subgroups were identified in 87 women. Each line represents the individual eGFR of a single participant. Each participant was allocated to the group of highest probability derived from the growth mixture model. Coefficients for the three groups of men: preserved and stable eGFR (n=213; intercept [mean eGFR at baseline], 113.3 ml/min per 1.73 m^2; 95% confidence interval [95% CI], 111.3 to 115.3; slope [mean eGFR decline over time], −0.6 ml/min per 1.73 m^2 per year; 95% CI, 0.0 to −0.9); rapid decline in eGFR (n=25; intercept, 109.5 ml/min per 1.73 m^2; 95% CI, 99.1 to 119.9; slope, −18.2 ml/min per 1.73 m^2 per year; 95% CI, −13.5 to −22.9); and baseline dysfunction (n=25; intercept, 55.6 ml/min per 1.73 m^2; 95% CI, 48.5 to 62.7; slope, −3.8 ml/min per 1.73 m^2 per year; 95% CI, −0.7 to −6.9). Coefficients for the two groups women: preserved and stable eGFR (n=84; intercept, 120.5 ml/min per 1.73 m^2; 95% CI, 118.1 to 122.9; slope, −0.6 ml/min per 1.73 m^2 per year; 95% CI, 0.2 to −1.4). We also identified a small number with rapid decline in kidney function (n=3; intercept, 127.5 ml/min per 1.73 m^2; 95% CI, 119.3 to 135.7; slope, −14.6 ml/min per /1.73 m^2 per year; 95% CI,−7.5 to −21.7).

trajectory in men using the preserved and stable trajectory as the reference are presented in Table 2. Outdoor work (OR, 10.35; 95% CI, 1.35 to 79.24), (nonsugarcane) agricultural work (OR, 3.57; 95% CI, 1.14 to 11.13), and lack of shade available during work breaks (OR, 3.74; 95% CI, 1.59 to 8.76) were associated with this outcome. However, we found no evidence for associations between rapid decline and years of work in sugarcane or agriculture; self-reported physical

Table 2. Age- and education level–adjusted associations of rapid decline in eGFR by baseline exposure in study participants who were men

Characteristic	Rapid Decline in eGFR[a]	
	OR	95% CI
Alcohol consumption		
Any	1.69	0.70 to 4.10
None	Reference	Reference
NSAID use		
Daily/regularly	1.28	0.34 to 4.74
Never/occasionally	Reference	Reference
Water sources		
Piped water	0.79	0.34 to 1.81
Dug well/drilled well	Reference	Reference
Water hardness		
Soft/moderately hard	1.21	0.47 to 3.11
Hard/very hard	Reference	Reference
Total liquid in last 24 h, L		
>5.0	1.01	0.43 to 2.38
≤5.0	Reference	Reference
Current occupation		
Sugarcane	1.51	0.31 to 7.29
Agricultural work	3.57	1.14 to 11.13
Other occupations/EIP	Reference	Reference
Main sugarcane role (if ever worked in sugarcane)		
Cane/seed cutter	2.15	0.57 to 8.06
Seeder	1.82	0.40 to 8.20
Other cane jobs	0.94	0.14 to 6.08
Never worked in sugarcane	Reference	Reference
Current or historical banana work		
Yes	1.77	0.60 to 5.18
No	Reference	Reference
Years in sugarcane	1.02	0.87 to 1.19
Years in agriculture	0.99	0.89 to 1.09
Work carried out[b]		
Outdoors	10.35	1.35 to 79.24
Indoors	Reference	Reference
Work in a hot environment[b]		
Regular/frequently	0.46	0.20 to 1.06
Irregularly	Reference	Reference
Shade availability[b]		
No	3.74	1.59 to 8.76
Yes or inside	Reference	Reference
Duration of breaks,[b] min		
≤10	1.86	0.80 to 4.33
>10	Reference	Reference
Physical effort at work[c]		
Moderate/hard	1.40	0.59 to 3.32
None/slight	Reference	Reference
Agrochemicals[b,d]		
Yes	1.70	0.72 to 4.03
No	Reference	Reference
Heat/dehydration symptoms[b]		
Yes	1.40	0.55 to 3.55
No	Reference	Reference
Dysuria[b]		
Yes	1.18	0.48 to 2.89
No	Reference	Reference

Table 2. Continued

Characteristic	Rapid Decline in eGFR[a]	
	OR	95% CI
Fever[b]		
Yes	2.41	0.80 to 7.27
No	Reference	Reference

Agricultural work includes all nonsugarcane agricultural work. OR, odds ratio; 95% CI, 95% confidence interval; NSAID, nonsteroidal anti-inflammatory drug; EIP, economically inactive population.
[a]Rapid decline versus preserved and stable eGFR. Probability weighted according to the results of the growth mixture model.
[b]Over the last 6 months.
[c]Over the last week.
[d]Data were collected at the second visit and included glyphosate, cypermethrin, paraquat, and methomyl.

effort in the last week at work; self-reported occupational heat or agrochemical exposure over last 6 months; alcohol consumption, self-reported fluid consumption, or water quality or source; heat/dehydration-related symptoms; or use of nonsteroidal anti-inflammatory drugs.

We were concerned that the questionnaire administered at baseline might fail to capture important occupational exposures, because for most participants, it was conducted 6 months after the harvest season. Therefore, we conducted a sensitivity analysis (men recruited at the November visit only; n=213) examining the association with the same rapid decline eGFR trajectory as above and occupational exposures, hydration variables, and heat-related symptoms captured at the second study visit (May 2015; immediately after harvest) (Supplemental Table 4). At this time point, no associations were detected between working outdoors or lack of shade and rapid decline in eGFR trajectory (although very few participants were not exposed). There was an association between both those working in a sugarcane cutting role (OR, 3.84; 95% CI, 1.17 to 12.58) and those reporting fever over the last 6 months (OR, 5.77; 95% CI, 2.03 to 16.33) and rapid decline trajectory, but in line with the baseline exposure analysis, no associations were observed between self-reported measures of heat exposure, combined heat-related symptoms, or fluid intake and outcome (Supplemental Tables 5 and 6).

Urinary Findings

No associations were found between dipstick proteinuria, specific gravity, or ACR and eGFR trajectory subgroups (Tables 3 and 4). Urinary NGAL levels among men differed between the three groups tested (Figure 4). The positive and negative predictive values of NGAL≥5.5 pg/mmol for rapid decline were 28.5% and 62.5%, respectively.

DISCUSSION

This is the first community-based cohort study from an area with high reported prevalence of MeN and the first longitudinal study of at least moderate size with follow-up of >6 months in an area at high risk of disease. Even after excluding those with self-reported CKD, 9.5% of the apparently healthy men (but no women) in the study had evidence of baseline renal dysfunction. Rapid loss of eGFR from normal baseline levels was found in another 9.5% of men and 3.4% of women. Among men, risk factors at baseline for rapid decline included working outdoors, agricultural work, and lack of shade availability, but none of the other questionnaire responses aimed at capturing heat stress, time-accumulated occupation, or other proposed causes of MeN were associated with the outcome at baseline. Because of small numbers, we were unable to examine associations in women.

Other important findings from our study include the cyclical annual fluctuation in renal function across the entire population, with the average eGFR approximately 2.5-ml/min per 1.73 m^2 lower after the dry (harvest) season compared with 6 months earlier. Furthermore, although there were no differences in albuminuria between those with different kidney function trajectories, urinary NGAL was substantially higher among those with baseline dysfunction and marginally elevated in the rapid decline group.

Although CKDu has been anecdotally reported as an aggressive disease,[1] the rate of loss of kidney function in those in the rapid decline group who make up almost 10% of the unselected population of young men in our study is, to our knowledge, without precedent. Even compared with eGFR decline in other forms of CKD seen in clinic populations, the observed loss of kidney function is alarming. Although a recent biopsy study that enrolled patients with established CKDu reported a rate of decline in eGFR of 7.0 ml/min per 1.73 m^2 per year among men with a history of work in the sugarcane,[24] there have been no longitudinal studies that have examined medium- or long-term (>1-year) changes in kidney function in the at-risk population. The rate of eGFR decline has been explored in more detail in other forms of CKD; for example, a longitudinal study in 55 clinic patients with diabetic nephropathy from Belgium reported that approximately 15% of patients suffered severe decline in kidney function (defined as eGFR loss >4 ml/min per 1.73 m^2 per year).[25] Most recently, Boucquemont *et al.*[26] examined eGFR decline in a patient population with CKD in France using a similar latent class-based modeling approach to that used in this analysis. This study reported severe eGFR decline in only 0.6% of patients (approximately

Table 3. Description of urinary findings at baseline by assigned eGFR trajectory groups in men

Urine Findings	Overall, *n*=263	Preserved and Stable eGFR, *n*=213	Rapid Decline in eGFR, *n*=25	Baseline Dysfunction, *n*=25
Urinary specific gravity, *n* (%)				
≤1020	256 (97.3)	207 (97.2)	24 (96.0)	25 (100.0)
>1020	7 (2.7)	6 (2.8)	1 (4.0)	0 (0)
Protein, *n* (%)				
Negative	224 (85.2)	181 (85.0)	22 (88.0)	21 (84.0)
Trace	25 (9.5)	19 (8.9)	2 (8.0)	4 (16.0)
Positive	14 (5.3)	13 (6.1)	1 (4.0)	0 (0)
ACR, mg/g, *n* (%)				
≥30	15 (5.7)	11 (5.2)	0 (0)	4 (16.0)
<30	248 (94.3)	201 (94.8)	25 (100.0)	21 (84.0)

Participants were assigned to the group with the highest probability in the growth mixture model. *P* values were NS by Fishers exact test for differences by group. ACR, albumin-to-creatinine ratio.

50 ml/min per 1.73 m^2 over almost 6 years). Therefore, our study findings underline the unique and severe nature of kidney disease in this region.

The associations with rapid decline trajectory in men suggest that occupation (outdoor agricultural work) is an important risk factor for loss of kidney function, consistent with previous reports.[18] The temporary nature of work in this population makes distinguishing relationships between specific occupations and eGFR loss challenging; however, it is interesting to note that neither time-accumulated sugarcane work nor agricultural work were associated with outcome. Furthermore, the association between lack of available shade at baseline and rapid decline trajectory suggests that working environment may play an important role in disease evolution either by (not reducing) solar exposure or as a surrogate for generally poor occupational conditions. Consistent with this and in line with previous crossharvest studies,[17] we identified an association between rapid decline and a cane/seed cutting role (a job role that has been associated with particularly hot working conditions) in a sensitivity analysis examining associations with exposures assessed postharvest.

The abscence of an association between variables aimed at capturing heat stress (self-reported physical effort the previous week at work and both work in very hot environment and combined dehydration/heat stress symptoms in the last 6 months) and the outcome measure, both at baseline and in the sensitivity analysis with exposures assessed at visit 2 raises further questions. Although self-reported measures of thermal sensation and physical exertion have been shown to robustly capture acute physiologic heat stress,[27] our (similar) instruments (and/or our combined measure of heat symptoms) may not be valid in the rural Nicaraguan population, or they may not reflect time-accumulated heat stress. Alternatively, we may have had inadequate power to detect heat stress as a partial contributor to eGFR decline, or otherwise, it may be that nonheat-related occupational exposures promote the development of CKDu. Finally, the association between self-reported fever over the previous 6 months at the second study visit and the rapid decline trajectory might support a proposed infective/inflammatory contributor to MeN,[28] although this finding was from a sensitivity analysis and should be treated with caution.

In summary, our data do not provide clear evidence for a cause of the disease. Along with occupation, the importance of nonoccupational factors is supported by (*1*) the range of jobs undertaken by the men experiencing rapid decline and (*2*) the 3.4% of women in our study who also showed a rapid loss of eGFR. As others have suggested,[2] separate initiating

Table 4. Description of urinary findings at baseline by assigned eGFR trajectory groups in women

Urine Findings	Overall, *n*=87	Preserved and Stable eGFR, *n*=84	Rapid Decline in eGFR, *n*=3
Urinary specific gravity, *n* (%)			
≤1020	81 (93.1)	79 (94.1)	2 (66.7)
>1020	6 (6.9)	5 (5.9)	1 (33.3)
Protein, *n* (%)			
Negative	70 (80.5)	68 (81.0)	2 (66.7)
Trace	13 (14.9)	12 (14.3)	1 (33.3))
Positive	4 (4.6)	4 (4.7)	0 (0)
ACR, mg/g, *n* (%)			
≥30	9 (10.3)	9 (10.7)	0 (0)
<30	78 (89.7)	75 (89.3)	3 (100.0)

Participants were assigned to the group with the highest probability in the growth mixture model. Given the small number in some cells, no statistical tests were performed. ACR, albumin-to-creatinine ratio.

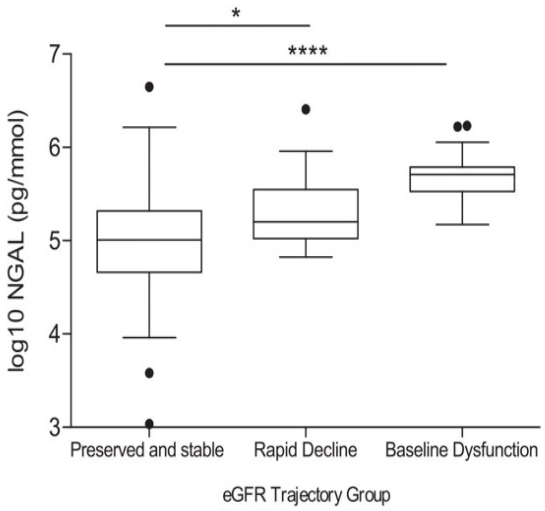

Figure 4. Urinary NGAL concentrations were higher in men with baseline kidney dysfunction and those who experienced rapid decline in eGFR. Box and whisker plot of urinary neutrophil gelatinase–associated lipocalin (NGAL)/creatinine concentrations by assigned eGFR trajectory group among male study participants. Lines indicate medians. Boxes are interquartile ranges. Whiskers indicate 1.5× interquartile ranges. Dots are outlying values. Stable group, n=55; rapid decline group, n=25; baseline dysfunction, n=24. *P=0.03 using ANOVA followed by the Dunnett multiple comparisons test (using the stable and preserved eGFR group as the reference); ****$P \leq 0.001$ using ANOVA followed by the Dunnett multiple comparisons test (using the stable and preserved eGFR group as the reference).

and exacerbating factors should be considered, such as in other forms of CKD. For example, the progression of kidney disease due to known causes (*e.g.*, diabetes or GN) can be exacerbated by episodes of volume depletion. Therefore, the possibility of an initial (currently unknown) subclinical insult, which is then exacerbated by the harsh working conditions, might explain the increased rates of eGFR loss and excess of advanced disease in men.

Although other studies have identified changes in urinary biomarkers in sugarcane workers over the harvest season in Mesoamerica,[29] none have examined associations with subsequent eGFR loss over the medium term. There were no associations between dipstick proteinuria or ACR and eGFR trajectory group. Although albuminuria is a strong risk factor for renal decline in most populations, this is consistent with previous reports from Mesoamerica, where patients with established CKDu show only low-grade proteinuria.[6,24,30] Urinary NGAL levels were substantially raised in those with baseline dysfunction, but levels in the rapid decline group overlapped with the stable group, making this test poorly predictive at an individual level.

Finally, it is worth noting the seasonal variation of eGFR in the population. Other studies (unrelated to CKDu) have described similar seasonal differences in renal function[31,32]; whether this variation is in any way related to the factors that cause MeN is unclear, but this finding does need to be considered when interpreting the change in eGFR reported in crossharvest studies.[5,18] Ideally, any future longitudinal biomarker study should be of >1 year in duration to ensure that small falls in eGFR do not reflect cyclical seasonal changes.

Our study has several strengths. Overall response rates were high, and the eGFR was estimated using robust methods. We excluded those with self-report of diabetes and hypertension in an attempt to focus our study on eGFR decline due to MeN, and the prospective nature of our study enabled us to identify those with aggressive disease without necessarily meeting definitions for CKD. Furthermore, we excluded those with established renal disease (either by self-report from the study as a whole or by examining only those with preserved eGFR at baseline for the risk factor analysis), and hence, we could overcome issues associated with reverse causation.

Our study also has limitations. We did not formally exclude diabetes in our participants. Although often undiagnosed,[33] the prevalence of diabetes is low in Nicaraguans of this age group,[34] and none of those in the rapid decline group showed albuminuria (or glycosuria; data not presented), making an underlying diabetic lesion highly unlikely. We also relied on self-report to quantify the majority of occupational and environmental exposures. Although questionnaire-based assessments are useful instruments, none of them have been validated in the Nicaraguan population; therefore, some exposures may be prone to misclassification. The study took place in a confined geographical area, which limits generalizability. Resources restricted our study to a moderate sample size, and we had to alter our statistical approach. We were nonetheless able to detect a number of strong associations with eGFR trajectory, but the analytical change did lead to a reduction in power. Therefore, we would have expected to identify associations with a primary cause of disease that had been reliably captured by questionnaire but may have missed weaker associations, particularly with contributing exposures. The baseline dysfunction group is unrepresentative due to selection criteria (those with established CKD were intentionally excluded at recruitment) and possibly survivor bias (due to the small number of deaths in this group), and the nature of the study design means that the relationship between rapid decline in eGFR and hard outcomes could not be described. However, we hope to perform extended follow-up to investigate the longer-term outcomes in the cohort. Finally, the CKD-EPI formula has not been validated for this population, although because we were interested in within-person change in eGFR, this is unlikely to be of major importance.

In conclusion, this is the first community-based cohort study that describes the natural history of eGFR in those at risk of MeN. Almost 10% of apparently healthy young men and 3.4% of young women showed a marked decline in kidney function. Additional studies with at least 1-year of follow-up are needed to understand the causes of this decline, including the risks associated with outdoor (agricultural) work. Efforts to identify biomarkers of this early loss of eGFR rather than established disease are essential to gain a better understanding

of etiology as well as to identify the population(s) that would benefit from interventions. A combined multidisciplinary approach is called for in partnership with the affected communities and local employers to address this devastating disease.

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DISCLOSURES

None.

REFERENCES

1. Ordunez P, Saenz C, Martinez R, Chapman E, Reveiz L, Becerra F: The epidemic of chronic kidney disease in Central America. *Lancet Glob Health* 2: e440–e441, 2014
2. Wegman D, Crowe J, Hogstedt C, Jakobsson K, Wesseling C: Mesoamerican nephropathy: Report from the second international research workshop on MeN. In: SALTRA, Vol. 33: 1–193, edited by Central American Institute for Studies on Toxic Substances (IRET-UNA) and Program on Work, Environment and Health in Central America, Heredia, Costa Rica, SALTRA/IRET-UNA, 2016
3. Orantes CM, Herrera R, Almaguer M, Brizuela EG, Núñez L, Alvarado NP, et al.: Epidemiology of chronic kidney disease in adults of Salvadoran agricultural communities. *MEDICC Rev* 16: 23–30, 2014
4. Ordunez P, Martinez R, Reveiz L, Chapman E, Saenz C, Soares da Silva A, et al.: Chronic kidney disease epidemic in Central America: Urgent public health action is needed amid causal uncertainty. *PLoS Negl Trop Dis* 8: e3019, 2014
5. Peraza S, Wesseling C, Aragon A, Leiva R, García-Trabanino RA, Torres C, et al.: Decreased kidney function among agricultural workers in El Salvador. *Am J Kidney Dis* 59: 531–540, 2012
6. Torres C, Aragón A, González M, López I, Jakobsson K, Elinder CG, et al.: Decreased kidney function of unknown cause in Nicaragua: A community-based survey. *Am J Kidney Dis* 55: 485–496, 2010
7. Jayatilake N, Mendis S, Maheepala P, Mehta FR; CKDu National Research Project Team: Chronic kidney disease of uncertain aetiology: Prevalence and causative factors in a developing country. *BMC Nephrol* 14: 180, 2013
8. Ministry of Health Nutrition and Indigenous Medicine Medical Statistics Unit: *Annual Health Bulletin of Sri Lanka 2015*, Colombo, Sri Lanka, Ministry of Health, Nutrition and Indigenous Medicine, 2017
9. Wijkström J, Jayasumana C, Dassanayake R, Priyawardane N, Godakanda N, Siribaddana S, et al.: Morphological and clinical findings in Sri Lankan patients with chronic kidney disease of unknown cause (CKDu): Similarities and differences with Mesoamerican Nephropathy. *PLoS One* 13: e0193056, 2018
10. Rajapurkar MM, John GT, Kirpalani AL, Abraham G, Agarwal SK, Almeida AF, et al.: What do we know about chronic kidney disease in India: First report of the Indian CKD registry. *BMC Nephrol* 13: 10, 2012
11. El Minshawy O, Ghabrah T, El Bassuoni E: End-stage renal disease in Tabuk Area, Saudi Arabia: An epidemiological study. *Saudi J Kidney Dis Transpl* 25: 192–195, 2014
12. Correa-Rotter R, Wesseling C, Johnson RJ: CKD of unknown origin in Central America: The case for a Mesoamerican nephropathy. *Am J Kidney Dis* 63: 506–520, 2014
13. Riefkohl A, Ramírez-Rubio O, Laws RL, McClean MD, Weiner DE, Kaufman JS, et al.: Leptospira seropositivity as a risk factor for Mesoamerican Nephropathy. *Int J Occup Environ Health* 23: 1–10, 2017
14. Valcke M, Levasseur ME, Soares da Silva A, Wesseling C: Pesticide exposures and chronic kidney disease of unknown etiology: An epidemiologic review. *Environ Health* 16: 49, 2017
15. O'Donnell JK, Tobey M, Weiner DE, Stevens LA, Johnson S, Stringham P, et al.: Prevalence of and risk factors for chronic kidney disease in rural Nicaragua. *Nephrol Dial Transplant* 26: 2798–2805, 2011
16. Wesseling C, Aragón A, González M, Weiss I, Glaser J, Rivard CJ, et al.: Heat stress, hydration and uric acid: A cross-sectional study in workers of three occupations in a hotspot of Mesoamerican nephropathy in Nicaragua. *BMJ Open* 6: e011034, 2016
17. Laws RL, Brooks DR, Amador JJ, Weiner DE, Kaufman JS, Ramírez-Rubio O, et al.: Changes in kidney function among Nicaraguan sugarcane workers. *Int J Occup Environ Health* 21: 241–250, 2015
18. Wesseling C, Aragón A, González M, Weiss I, Glaser J, Bobadilla NA, et al.: Kidney function in sugarcane cutters in Nicaragua–A longitudinal study of workers at risk of Mesoamerican nephropathy. *Environ Res* 147: 125–132, 2016
19. González-Quiroz M, Caplin B, Pearce N, Nitsch D: What do epidemiological studies tell us about chronic kidney disease of undetermined cause in Meso-America? A systematic review and meta-analysis. *Clin Kidney J*: sfx136, 2017
20. González-Quiroz M, Camacho A, Faber D, Aragón A, Wesseling C, Glaser J, et al.: Rationale, description and baseline findings of a community-based prospective cohort study of kidney function amongst the young rural population of Northwest Nicaragua. *BMC Nephrol* 18: 16, 2017
21. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al.; CKD-EPI Investigators: Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med* 367: 20–29, 2012
22. Boucquemont J, Heinze G, Jager KJ, Oberbauer R, Leffondre K: Regression methods for investigating risk factors of chronic kidney disease outcomes: The state of the art. *BMC Nephrol* 15: 45, 2014
23. Nylund KL, Asparouhov T, Muthén BO: Deciding on the number of classes in latent class analysis and growth mixture modeling: A Monte Carlo simulation study. *Struct Equ Modeling* 14: 535–569, 2007
24. Wijkström J, González-Quiroz M, Hernandez M, Trujillo Z, Hultenby K, Ring A, et al.: Renal morphology, clinical findings, and progression rate in mesoamerican nephropathy. *Am J Kidney Dis* 69: 626–636, 2017
25. Goderis G, Van Pottelbergh G, Truyers C, Van Casteren V, De Clercq E, Van Den Broeke C, et al.: Long-term evolution of renal function in patients with type 2 diabetes mellitus: A registry-based retrospective cohort study. *BMJ Open* 3: e004029, 2013
26. Boucquemont J, Loubère L, Metzger M, Combe C, Stengel B, Leffondre K; NephroTest Study Group: Identifying subgroups of renal function trajectories. *Nephrol Dial Transplant* 32[Suppl 2]: ii185–ii193, 2017
27. Chan AP, Yang Y: Practical on-site measurement of heat strain with the use of a perceptual strain index. *Int Arch Occup Environ Health* 89: 299–306, 2016
28. Fischer RSB, Vangala C, Truong L, Mandayam S, Chavarria D, Granera Llanes OM, et al.: Early detection of acute tubulointerstitial nephritis in the genesis of Mesoamerican nephropathy. *Kidney Int* 93: 681–690, 2018
29. Laws RL, Brooks DR, Amador JJ, Weiner DE, Kaufman JS, Ramírez-Rubio O, et al.: Biomarkers of kidney injury among nicaraguan sugarcane workers. *Am J Kidney Dis* 67: 209–217, 2016

30. Gracia-Trabanino R, Domínguez J, Jansà JM, Oliver A: [Proteinuria and chronic renal failure in the coast of El Salvador: Detection with low cost methods and associated factors]. *Nefrologia* 25: 31–38, 2005
31. Masugata H, Senda S, Inukai M, Himoto T, Murao K, Hosomi N, et al.: Seasonal variation in estimated glomerular filtration rate based on serum creatinine levels in hypertensive patients. *Tohoku J Exp Med* 224: 137–142, 2011
32. Ranucci M, Castelvecchio S, La Rovere MT; Surgical and Clinical Outcome Research (SCORE) Group: Renal function changes and seasonal temperature in patients undergoing cardiac surgery. *Chronobiol Int* 31: 175–181, 2014
33. Barcelo A, Gregg EW, Gerzoff RB, Wong R, Perez Flores E, Ramirez-Zea M, et al.; CAMDI Collaborative Study Group: Prevalence of diabetes and intermediate hyperglycemia among adults from the first multinational study of noncommunicable diseases in six Central American countries: The Central America Diabetes Initiative (CAMDI). *Diabetes Care* 35: 738–740, 2012
34. Iniciativa Centroamericana de Diabetes (CAMDI): *Encuesta de Diabetes, Hipertensión y Factores de Riesgo de Enfermedades Crónicas*, Villa Nueva, Guatemala, Organización Panamericana de la Salud, 2009

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4.5. Supplementary material

1. Supplementary Methods
2. Regulatory approval reference numbers
3. References
4. Supplementary Tables
    a. Supplementary Table 1: Symptoms reported over the last 6 months that were reported at baseline
    b. Supplementary Table 2: Baseline demographic and lifestyle characteristics of male study participants stratified by assigned eGFR trajectory group
    c. Supplementary Table 3: Baseline demographic and lifestyle characteristics of female study participants stratified by assigned eGFR trajectory group
    d. Supplementary Table 4. Occupational characteristics, heat symptoms and liquid intake at visit 2 for males recruited at the first study visit only, stratified by assigned eGFR trajectory group
    e. Supplementary Table 5. Age- and education level-adjusted associations of the rapid decline trajectory with exposures at visit 2 among male study participants
    f. Supplementary Table 6: Age- and education level-adjusted associations of baseline kidney dysfunction with baseline exposures in male study participants
    g. Supplementary Table 7: Age- and education level-adjusted multivariate analysis of associations of baseline exposures with changes in eGFR over the follow-up period in the male population.

5. Supplementary Figures
    a. Supplementary Figure 1: Distribution of eGFR trajectories in the study population.
    b. Supplementary Figure 2: Serum creatinine levels in the different eGFR trajectory groups over time.

1. Supplementary Methods

Communities and population: The study was conducted in 9 rural communities in the Leon and Chinandega regions of northwest Nicaragua. Most members of these communities had not previously participated in CKD research studies, but no one had participated in previous studies. Following engagement activities, the study team enumerated the de jure population aged 18-30 years in each village by visiting dwellings and liaising with families and community leaders. A comparison with current local government data suggests that the sampling frame n=520 represented 10-15% of the total population (including children) of the area. Internal and external migration indicates that numbers of adults in this age range are relatively underrepresented in these rural communities.

Questionnaire data, clinical measures and water samples: We collected exposure variables using a questionnaire, the details of which are available elsewhere.[1] In addition, at the second visit, we also asked participants to report any use of glyphosate, paraquat, methomyl, and cypermethrin with assistance from a visual catalogue.

Questionnaires collected the following data at baseline and each study visit:

A. Demographics: age, education, household income per month, water sources, and social security status.
B. Occupational history: current occupation, previous history of sugarcane work, predominant sugarcane job if the individual ever worked in the sugarcane industry, current and historical banana work, previous job duration, and number of sugarcane harvests or pre-harvests worked.
C. Current occupational exposures: daily work hours, duration of breaks, location of work, experience of a hot working environment, availability of shade during work breaks, physical effort last week at work, weight loss at work.
D. Lifestyle factors: grams of alcohol consumed over the last year, smoking in pack-years, liquid (water and soft drinks) intake in the last 24 hours, and use of nephrotoxic medications at any time.
E. Self-reported symptoms in the last 6 months, which were separated into the following categories: (i) heat-related/dehydration symptoms: headache, tachycardia, muscle cramps in the arms or legs, fever, nausea, difficulty breathing, muscle weakness, dizziness, vomiting, fainting, dysuria, dry mouth, very dark urine, very little urine, extreme tiredness and confusion;[2-4] (ii) self-reported weight loss; (iii) self-reported diagnoses of urinary tract infections in the previous year. Original symptom questionnaire responses for the whole population are summarized in Supplementary Table 1.

Height, weight and blood pressure were measured using standard procedures and calibrated devices.

Water was collected from participants' primary water source at baseline and the second visit only. Water was collected and stored in polypropylene containers at 4°C. Water hardness was calculated from the sum of concentrations of calcium and magnesium cations (as calcium carbonate, in mg/L), which were measured using inductively coupled plasma mass spectrometry at Imperial College London.

Urine and serum markers: All biosamples were stored at 4°C upon collection, frozen at the end of the study day (-20°C, for up to 2 weeks), and then stored at -80°C following transfer to the UK. A dipstick (Siemens Multistix, SG10) analysis was performed on previously frozen urine samples to semi-quantitatively assess protein concentrations and specific gravity. Albumin levels were quantified using a colorimetric method with a bromocresol green Albumin Assay Kit (Sigma-Aldrich, MAK124). Baseline urine samples from men that had been previously frozen and stored at -80°C for 2.5 years were analysed for neutrophil gelatinase-associated lipocalin (NGAL) levels. Samples from fifty-five individuals in the stable kidney function group were randomly selected, and samples from all individuals displaying a rapid decline in kidney function or who exhibited established renal dysfunction at baseline were examined. NGAL levels were measured using enzyme-linked immunoassay (ELH-Lipocalin2, RayBiotech) according to the manufacturer's instructions. Albumin and NGAL levels are reported as ratios to urinary creatinine levels measured using the Jaffe method (Sigma-Aldrich, C4255).

Serum creatinine (Scr) and cystatin C (Scys) levels were both measured in a single batch analysis of all 1581 stored study samples at the Nuffield Department of Population Health Wolfson Laboratories from the Clinical Trials Services Unit (CTSU), Oxford University using quality control referenced to isotope dilution mass spectrometry traceable international standards. The Scr levels were quantified using a Beckman AU680 Chemistry Analyser (Jaffe compensated method) and Scys levels were measured using a Siemens BN ProSpec (nephelometry). Mean expanded uncertainty values were: 3% at both 2.2 mg/dL and 4.7 mg/dL for Scr; and 8% at 0.7 mg/L and 6% at 2.1 mg/L for Scys. The CKD EPI formula for serum creatinine - cystatin C levels was then used to estimate the GFR.

Exposure variables: The original frequencies of self-reported symptoms (prior to recording) are presented in Supplementary Table 1. Prior to association analyses, income was recorded using the World Bank definition of poverty of an income of US $1.90/day and education level of illiteracy or attendance at primary-level education versus attendance at secondary- or higher-level education. Current occupation was grouped into sugarcane work, other agricultural work, and other occupations/economically inactive population; the predominant sugar cane role was categorized by potential heat exposure as (cane/seed) cutters, seeders or other roles. Work breaks categorized as <10 minutes or >10 minutes; self-reported occupational agrochemical exposure was reported as the use of any of glyphosate, paraquat, cypermethrin, or methomyl; and the degree of physical effort in last week at work was reported as none or slight versus moderate or hard. Lifestyle risk factors included daily average

alcohol consumption over the last year, which was defined as none (0 grams for both sexes) versus any (1-60 grams for males and 1-40 grams for females), and current smoking status classified as never smokers (0 pack-years) or light smokers (1-20 pack-years). Non-steroidal anti-inflammatory drug use was recorded as daily or regularly versus occasionally or never. The self-reported diagnosis of urinary tract infections was also recorded. Self-reported symptoms were categorized as positive for any one heat-related/dehydration symptom, along with fever, dysuria and self-reported weight loss.

Three variables related to fluid consumption were analysed: water sources (piped water or water from a dug well/drilled well) and total liquid intake in last 24 hours (≤5 litres/day, and >5 litres/day). Water hardness was grouped based as soft/moderately hard (0-120 mg/L), and hard/very hard (≥121 mg/L).

Additional statistical methods

Continuous variables were summarized as means and standard deviations or medians and interquartile ranges, and categorical variables are reported as frequencies/percentages. For further analyses, non-normally distributed continuous variables were categorized, and ordinal variables were recorded to avoid small cell numbers (see Supplementary Table 1).

We found that a multivariate linear model exhibited a poor fit with non-normally distributed residuals, as eGFR trajectories clustered in sub-populations and differed between men and women. Therefore, we used a growth mixture modelling (GMM) approach. We identified three subpopulations of eGFR

trajectories in men and two in women, primarily using the Bayesian Information Criterion suggested for this setting.[5] Adjustments for the season of follow-up visit did not substantially affect the estimation of eGFR trajectories and thus were not included in the model.

Associations between individual exposure variables and outcomes were examined using probability-weighted multinomial logistic regression analysis. Individuals' probabilities of each kidney function status were obtained from the GMM. These models were adjusted for age and educational level, as these variables might confound the associations between casual factors of interest and outcomes. The 95% confidence intervals of odds ratios that did not include unity were accepted as significant. As these association models consisted of exploratory analyses, no formal adjustments for multiple testing were performed.

The original study sample of 350 individuals was calculated to achieve 90% power to detect associations with an acceleration of the decrease in eGFR of 5.0 mL/min/1.73 m^2/year in a linear model to which at least 20% of the population were exposed at an alpha of 0.01. Given the change in analytical strategy, we performed a post hoc power calculation. Using a chi-squared test to detect differences in kidney function status (e.g., stable versus rapid decline) associated with an exposure (to which half the population are exposed), a greater than 80% power would detect an odds ratio of 3.8 or greater. With a non-exposed prevalence of 4.5%, this value reflects a similar power to detect an exposure that multiplies the risk by 3.3. This estimate is conservative, as the

analysis was based on a probability-weighted kidney function status, an outcome that is a more precise measure of the disease spectrum than an absolute categorical grouping.

The coefficients for exposures from the poorly fitted multivariate model, in which eGFR measures clustered within participants, including a random intercept and slope, are included as Supplementary Table 7 (for information purposes).

Analyses were performed using Stata v14 (Stata Corp.), Prism v7 (GraphPad Software) and Mplus (Muthén & Muthén) software.

2. Ethical approval and consent to participate

All participants signed a written informed consent form to participate in the follow-up study, in accordance with the Declaration of Helsinki. The study was approved by the Bioethical Review Board at the Medical Faculty of UNAN-León (Ref: FWA00004523/IRB00003342) and the Research Ethics Committee of the London School of Hygiene and Tropical Medicine (Ref: 8643/14363) in 2014.

3. Supplementary References

1. Gonzalez-Quiroz, M.*, et al.* Rationale, description and baseline findings of a community-based prospective cohort study of kidney function amongst the young rural population of Northwest Nicaragua. *BMC Nephrol* **18**, 16 (2017).
2. Crowe, J., Nilsson, M., Kjellstrom, T. & Wesseling, C. Heat-related symptoms in sugarcane harvesters. *Am J Ind Med* **58**, 541-548 (2015).
3. Departmen of the Army and Air Force. Technical bulletin: Heat stress control and heat causalty management (TB MED 507) Air Force Pamphlet 48-152(1). Vol. 1 (ed. Departmen of the Army and Air Force) 72 (Departmen of the Army and Air Force,, Washington, DC., 2003).

4. Ramirez-Rubio, O.*, et al.* Chronic kidney disease in Nicaragua: a qualitative analysis of semi-structured interviews with physicians and pharmacists. *BMC Public Health* **13**, 350 (2013).
5. Nylund, K.L., Asparouhov, T. & Muthén, B.O. Deciding on the Number of Classes in Latent Class Analysis and Growth Mixture Modeling: A Monte Carlo Simulation Study. *Structural Equation Modeling: A Multidisciplinary Journal* **14**, 535-569 (2007).

4. Supplementary Tables

Supplementary table 1: Symptoms reported over the last 6 months that were reported at baseline

Symptom	Overall (%) (*n*=350)	Males (*n*=263)	Females (*n*=87)
Heat-related/dehydration symptoms			
Headache (n, %)			
Yes	139 (39.7)	103 (39.2)	36 (41.4)
No	211 (60.3)	160 (60.8)	51 (58.6)
Tachycardia (n, %)			
Yes	40 (11.4)	27 (10.3)	13 (14.9)
No	310 (88.6)	236 (89.7)	74 (85.61)
Muscle cramps in the arms or legs (n, %)			
Yes	42 (12.0)	32 (12.2)	10 (11.5)
No	308 (88.0)	231 (87.8)	77 (88.5)
Muscle weakness (n, %)			
Yes	6 (1.7)	2 (0.8)	4 (4.6)
No	344 (98.3)	261 (99.2)	83 (95.4)
Fever (n, %)			
Yes	36 (10.3)	32 (12.2)	4 (4.6)
No	314 (89.7)	231 (87.8)	83 (95.4)
Nausea (n, %)			
Yes	25 (7.1)	22 (8.4)	3 (3.5)
No	325 (92.9)	241 (91.6)	84 (96.5)
Difficulty breathing (n, %)			
Yes	16 (4.6)	11 (4.2)	5 (5.8)
No	334 (95.4)	252 (95.8)	82 (94.2)
Dizziness (n, %)			
Yes	26 (7.4)	15 (5.7)	11 (12.6)
No	324 (92.6)	248 (94.3)	76 (87.4)
Vomiting (n, %)			
Yes	8 (2.3)	8 (3.0)	0 (0)
No	342 (97.7)	255 (97.0)	87 (100.0)
Fainting (n, %)			
Yes	7 (2.0)	5 (1.9)	2 (2.3)
No	343 (98.0)	258 (98.1)	85 (97.7)
Dysuria (n, %)			
Yes	94 (26.9)	72 (27.4)	22 (25.3)
No	256 (73.1)	191 (72.6)	65 (74.7)
Dry mouth (n, %)			
Yes	75 (21.4)	56 (21.3)	19 (21.8)
No	275 (78.6)	207 (78.7)	68 (78.2)
Very dark urine (n, %)			
Yes	21 (6.0)	12 (4.6)	9 (10.3)
No	329 (94.0)	251 (95.4)	78 (89.7)
Very little urine (n, %)			
Yes	30 (8.6)	16 (6.1)	14 (16.1)
No	320 (91.4)	247 (93.9)	73 (83.9)
Extremely tired (n, %)			
Yes	29 (8.3)	19 (7.2)	10 (11.5)
No	321 (91.7)	244 (92.8)	77 (88.5)
Confusion (n, %)			
Yes	12 (3.4)	8 (3.0)	4 (4.6)
No	338 (96.6)	255 (97.0)	83 (95.4)

Supplementary table 2: Baseline demographic and lifestyle characteristics of male study participants stratified by assigned eGFR trajectory group* (n=263)

Characteristic	Preserved and stable kidney function (*n*=213)	Rapid decline in eGFR (*n*=25)	Dysfunction at baseline (*n*=25)
Age, years; mean (SD)	23.6 (3.8)	23.3 (3.6)	25.4 (2.9)
Educational level; n (%)			
Illiteracy/primary	121 (56.8)	14 (56.0)	16 (64.0)
Secondary/higher	92 (43.2)	11 (44.0)	9 (36.0)
Body mass index; n, (%)			
Normal	176 (82.6)	18 (72.0)	21 (84.0)
Overweight/obese	37 (17.4)	7 (28.0)	4 (16.0)
Systolic blood pressure mmHg; median (IQR)	118 (110 – 124)	120 (113 – 127)	126 (119 – 129)
Diastolic blood pressure mmHg; median (IQR)	69 (63 – 73)	70 (66 – 76)	74 (68 – 78)
Income; n (%)			
Poor	108 (50.7)	16 (64.0)	14 (56.0)
Not poor	105 (49.3)	9 (36.0)	11 (44.0)
Family history of CKD; n (%)			
Yes	103 (48.4)	11 (44.0)	12 (48.0)
No	110 (51.6)	14 (56.0)	13 (52.0)
Alcohol consumption; n (%)			
Any	112 (52.6)	16 (64.0)	13 (52.0)
None	101 (47.4)	9 (36.0)	12 (48.0)
Smoking history; n (%)			
Light smokers	81 (38.0)	10 (40.0)	12 (48.0)
Never smokers	132 (62.0)	15 (60.0)	13 (52.0)
NSAID use; n (%)			
Daily-regularly	21 (9.9)	3 (12.0)	5 (20.0)
Never-occasionally	192 (90.1)	22 (88.0)	20 (80.0)
Water sources; n (%)			
Piped water	115 (54.0)	12 (48.0)	12 (48.0)
Dug well/drilled well	98 (46.0)	13 (52.0)	13 (52.0)
Water hardness; n (%)			
Soft/moderately hard	51 (23.9)	7 (28.0)	9 (36.0)
Hard/very hard	162 (76.1)	18 (72.0)	16 (65.0)
Total liquid intake in the last 24 hrs; n (%)			
≤ 5.0 litres	86 (40.4)	10 (40.0)	9 (36.0)
>5.0 litres	127 (59.6)	15 (60.0)	16 (64.0)
Current occupation; n (%)			
Sugarcane	38 (17.8)	3 (12.0)	4 (16.0)
Agricultural work	98 (46.0)	18 (72.0)	15 (60.0)
Other occupations/EIP	77 (36.2)	4 (16.0)	6 (24.0)
Main sugarcane role (if ever worked in sugarcane) (%)			
Cane/seed cutter	103 (48.4)	15 (60.0)	19 (76.0)
Seeder	39 (18.3)	5 (20.0)	3 (12.0)
Other cane jobs	30 (14.1)	2 (8.0)	1 (4.0)
Never worked in sugarcane	41 (19.3)	3 (12.0)	2 (8.0)
Current or historical banana work; n (%)			
Yes	27 (12.9)	5 (20.0)	15 (60.0)
No	186 (87.3)	20 (80.0)	10 (40.0)
Years in sugarcane; mean (SD)	2.7±2.9	2.7±2.7	3.6±2.8
Years in agriculture; mean (SD)	4.3±4.5	4.0±3.9	5.4±4.5
Work carried out; † n (%)			
Indoors	62 (29.1)	1 (4.0)	6 (24.0)
Outdoors	151 (70.9)	24 (96.0)	19 (76.0)
Work in a hot environment; † n (%)			
Irregularly	71 (33.3)	13 (52.0)	8 (32.0)
Regular/frequently	142 (66.7)	12 (48.0)	17 (68.0)
Shade availability; † n (%)			
Yes or inside	159 (74.7)	11 (44.0)	20 (80.0)
No	54 (25.3)	14 (56.0)	5 (20.0)
Duration of breaks; † n (%)			
≤ 10 minutes	63 (29.6)	11 (44.0)	12 (48.0)
>10 minutes	150 (70.4)	14 (56.0)	13 (52.0)

Characteristic	Preserved and stable kidney function (*n*=213)	Rapid decline in eGFR (*n*=25)	Dysfunction at baseline (*n*=25)
Physical effort at work; ‡ n (%)			
None/slight	94 (44.1)	9 (36.0)	11 (44.0)
Moderate/hard	119 (55.9)	16 (64.0)	14 (56.0)
Agrochemicals; †, § n (%)			
Yes	109 (51.2)	16 (64.0)	10 (40.0)
No	104 (48.8)	9 (36.0)	15 (60.0)
Heat/dehydration symptoms; † n (%)			
Yes	139 (65.3)	18 (72.0)	18 (72.0)
No	74 (34.7)	7 (28.0)	7 (28.0)
UTI diagnosis in the previous year; n (%)			
Yes	42 (19.7)	5 (20.0)	9 (36.0)
No	171 (80.3)	20 (80.0)	16 (64.0)
Weight loss; † n (%)			
Yes	42 (19.7)	7 (28.0)	6 (24.0)
No	171 (80.3	18 (72.0)	19 (76.0)
Dysuria †			
Yes	61 (28.6)	8 (32.0)	3 (12.0)
No	152 (71.4)	17 (68.0)	22 (88.0)
Fever†			
Yes	21 (9.9)	5 (20.0)	6 (24.0)
No	192 (90.1)	20 (80.0)	19 (76.0)
Baseline eGFR, mL/min/1.73 m^2; median (IQR)	117.9 (107.6 - 125.4)	116.5 (102.6 - 123.8)	55.5 (49.4 - 67.5)
Second eGFR, mL/min/1.73 m^2; median (IQR)	114.7 (104.5 - 122.3)	101.4 (91.2 - 108.9)	51.6 (44.6 - 64.6)
Third eGFR, mL/min/1.73 m^2; median (IQR)	115.3 (104.0 - 123.1)	103.0 (85.7 - 108.9)	55.0 (43.9 - 65.7)
Fourth eGFR, mL/min/1.73 m^2; median (IQR)	113.4 (105.5 - 121.6)	74.3 (62.7 - 84.3)	48.5 (39.0 - 56.8)
Final eGFR, mL/min/1.73 m^2; median (IQR)	113.7 (103.8 - 121.9)	77.1 (66.6 - 84.3)	53.2 (45.0 – 59.7)

*Abbreviations: CKD: chronic kidney disease; NSAID: non-steroidal anti-inflammatory drug; eGFR: estimated glomerular filtration rate using the CKD EPI equation based on creatinine and cystatin c levels; UTI: urinary tract infection. EIP: economically inactive population. Agricultural work includes all non-sugarcane agricultural work. *Participants assigned to the group with the highest probability in the growth mixture model. †Over the last 6 months; ‡over the last week; §data were collected at the second visit and include glyphosate, cypermethrin, paraquat and methomyl.*

Supplementary table 3: Baseline demographic and lifestyle characteristics of female study participants stratified by assigned eGFR trajectory group* (n=87)

Characteristic	Preserved and stable eGFR (*n*=84)	Rapid decline in eGFR (*n*=3)
Age, years; mean (SD)	24.3 ± 3.6	21.7 ± 3.0
Educational level; n (%)		
Illiteracy/primary	41 (48.8)	2 (66.7)
Secondary/higher	43 (51.2)	1 (33.3)
Body mass index; n, (%)		
Normal	46 (54.8)	1 (33.3)
Overweight/obese	38 (45.2)	2 (66.7)
Systolic blood pressure mmHg; median (IQR)	109 (102 – 117)	125 (103 – 133)
Diastolic blood pressure mmHg; median (IQR)	67 (63 – 72)	74 (67 – 75)
Income; n (%)		
Poor	50 (59.5)	1 (33.3)
Not poor	34 (40.5)	2 (66.7)
Family history of CKD; n (%)		
Yes	37 (44.1)	2 (66.7)
No	47 (55.9)	1 (33.3)
Alcohol consumption; n (%)		
Any	7 (8.3)	1 (33.3)
None	77 (91.7)	2 (66.7)
Smoking history; n (%)		
Light smokers	1 (1.2)	0 (0)
Never smokers	83 (98.8)	3 (100.0)
NSAID use; n (%)		
Daily-regularly	11 (13.1)	1 (33.3)
Never-occasionally	73 (86.9)	2 (66.7)
Water sources; n (%)		
Piped water	45 (53.6)	2 (66.7)
Dug well/drilled well	39 (46.4)	1 (33.3)
Water hardness; n (%)		
Soft/moderately hard	28 (33.3)	2 (66.7)
Hard/very hard	56 (66.7)	1 (33.3)
Total liquid intake in the last 24 hrs; n (%)		
≤ 5.0 litres	70 (83.3)	3 (100.0)
>5.0 litres	14 (16.7)	0 (0)
Current occupation; n (%)		
Sugarcane	10 (11.9)	0 (0)
Agricultural work	6 (7.1)	1 (33.3)
Other occupations/EIP	68 (81.0)	2 (66.7)
Main sugarcane role (if ever worked in sugarcane); n (%)		
Cane/seed cutter	0 (0)	0 (0)
Seeder	21 (25.0)	0 (0)
Other cane jobs	9 (10.7)	0 (0)
None	54 (64.3)	3 (100.0)
Current or historical banana work; n (%)		
Yes	9 (10.7)	0 (0)
No	75 (89.3)	3 (100.0)
Years in sugarcane; mean (SD)	0.6±1.7	0±0
Years in agriculture; mean (SD)	1.2±3.4	0.3±0.5
Work carried out; † n (%)		
Indoors	65 (77.4)	2 (66.7)
Outdoors	19 (22.6)	1 (33.3)
Work in a hot environment; † n (%)		
Irregularly	43 (51.2)	2 (66.7)
Regular/frequently	41 (48.8)	1 (33.3)
Shade availability; † n (%)		
Yes or inside	62 (73.8)	2 (66.7)
No	22 (26.2)	1 (33.3)
Duration of breaks; † n (%)		
≤ 10 minutes	14 (16.7)	1 (33.3)
>10 minutes	70 (83.3)	2 (66.7)
Physical effort at work; ‡ n (%)		
None/slight	42 (50.0)	1 (33.3)
Moderate/hard	42 (50.0)	2 (66.7)

Characteristic	Preserved and stable eGFR (*n*=84)	Rapid decline in eGFR (*n*=3)
Agrochemicals; †, § n (%)		
Yes	12 (14.3)	0 (0)
No	72 (85.7)	3 (100.0)
Heat/dehydration symptoms; † n (%)		
Yes	63 (75.0)	2 (66.7)
No	21 (25.5)	1 (33.3)
UTI diagnosis in the previous year; n (%)		
Yes	34 (40.5)	1 (33.3)
No	50 (59.5)	2 (66.7)
Weight loss; † n (%)		
Yes	8 (9.5)	0 (0)
No	76 (90.5)	3 (100.0)
Dysuria †		
Yes	21 (25.0)	1 (33.3)
No	63 (75.0)	2 (66.7)
Fever†		
Yes	4 (4.8)	0 (0)
No	80 (95.2)	3 (100.0)
Baseline eGFR, mL/min/1.73 m^2; median (IQR)	121.8 (115.8 – 127.1)	136.3 (123.3 – 136.4)
Second eGFR, mL/min/1.73 m^2; median (IQR)	118.9 (109.1 – 128.2)	107.5 (91.5 – 136.7)
Third eGFR, mL/min/1.73 m^2; median (IQR)	119.5 (113.0 – 126.6)	102.3 (93.1 – 108.2)
Fourth eGFR, mL/min/1.73 m^2; median (IQR)	119.4 (112.7 – 124.9)	99.9 (95.9 – 108.0)
Final eGFR, mL/min/1.73 m^2; median (IQR)	121.4 (112.1 – 126.6)	102.2 (102.0 – 103.1)

*Abbreviations: CKD: chronic kidney disease; NSAID: non-steroidal anti-inflammatory drug; eGFR: estimated glomerular filtration rate using the CKD EPI equation based on creatinine and cystatin c levels; UTI: urinary tract infection. EIP: economically inactive population. Agricultural work includes all non-sugarcane agricultural work. *Participants assigned to the group with the highest probability in the growth mixture model. †Over the last 6 months; ‡over the last week; §data were collected at the second visit and include glyphosate, cypermethrin, paraquat and methomyl.*

Supplementary table 4: Occupational characteristics, heat symptoms and liquid intake at visit 2 for males recruited at the first study visit only, stratified by assigned eGFR trajectory group* (n=213)

Characteristic	Preserved and stable eGFR (n=176)	Rapid decline in eGFR (n=18)	Dysfunction at baseline (n=19)
Total liquid intake in the last 24 hrs; n (%)			
≤ 5.0 litres	18 (10.2)	2 (11.1)	2 (10.5)
>5.0 litres	158 (89.8)	16 (88.9)	17 (89.5)
Current occupation; n (%)			
Sugarcane	89 (50.6)	10 (55.6)	5 (26.3)
Agricultural work	54 (30.7)	4 (22.2)	11 (57.9)
Other occupations/EIP	33 (18.7)	4 (22.2)	3 (15.8)
Predominant sugarcane role; † n (%)			
Cane/seed cutter	32 (18.1)	9 (50.0)	4 (21.1)
Seeder	33 (18.8)	4 (22.2)	0 (0)
Other cane jobs	39 (22.2)	0 (0)	4 (21.1)
Not worked in sugarcane	72 (40.9)	5 (27.8)	11 (57.9)
Months in sugarcane; † mean (SD)	2.9±2.7	4.2±2.5	1.2±2.2
Months in agriculture; † mean (SD)	1.6±2.3	0.4±1.4	3.0±2.5
Work carried out; † n (%)			
Indoors	39 (22.2)	3 (16.7)	5 (26.3)
Outdoors	137 (77.8)	15 (83.3)	14 (73.7)
Work in a hot environment; † n (%)			
Irregularly	36 (20.5)	4 (22.2)	7 (36.8)
Regular/frequently	140 (79.5)	14 (77.8)	12 (63.2)
Shade availability; † n (%)			
Yes or inside	161 (91.5)	17 (94.4)	18 (94.7)
No	15 (8.5)	1 (5.6)	1 (5.3)
Duration of breaks; † n (%)			
≤ 10 minutes	69 (39.2)	8 (44.4)	7 (36.8)
>10 minutes	107 (60.8)	10 (55.6)	12 (63.2)
Physical effort at work; ‡ n (%)			
Slight	60 (34.1)	5 (27.8)	4 (21.1)
Moderate/hard	116 (65.9)	13 (72.2)	15 (78.9)
Weight loss at work; † n (%)			
Yes	41 (23.3)	3 (16.7)	2 (10.5)
No	135 (76.7)	15 (83.3)	17 (89.5)
Heat/dehydration symptoms; † n (%)			
Yes	127 (72.2)	12 (66.7)	14 (73.7)
No	49 (27.8)	6 (33.3)	5 (26.3)
Dysuria †			
Yes	58 (32.9)	3 (16.7)	3 (15.8)
No	118 (67.1)	15 (83.3)	16 (84.2)
Fever†			
Yes	36 (20.4)	10 (55.6)	7 (36.8)
No	140 (79.6)	8 (44.4)	12 (63.2)

*Abbreviations: EIP: economically inactive population. Agricultural work includes all non-sugarcane agricultural work. *Participants assigned to the group with the highest probability in the growth mixture model. †Over the last 6 months; ‡over the last week.*

Supplementary table 5: Age- and education level-adjusted associations* of the rapid decline trajectory with exposures at visit 2 among in male study participants (n=213)

Characteristic	Preserved and stable eGFR (n=176)	Rapid decline in eGFR (n=18)	
	Reference	OR	95% CI
Alcohol consumption			
Any	1.00	1.04	0.38 to 2.78
None	1.00	Reference	Reference
Total liquid intake in the last 24			
≤ 5.0 litres	1.00	1.08	0.22 to 5.16
>5.0 litres	1.00	Reference	Reference
Current occupation			
Sugarcane	1.00	0.84	0.24 to 2.96
Agricultural work	1.00	0.59	0.13 to 2.59
Other occupations/EIP	1.00	Reference	Reference
Main sugarcane role†			
Cane/seed cutter	1.00	3.84	1.17 to 12.58
Seeder	1.00	1.59	0.38 to 6.52
Other cane Jobs	1.00	--	--
Not worked in sugarcane	1.00	Reference	Reference
Months in sugarcane†	1.00	1.20	0.98 to 1.46
Months in agriculture†	1.00	0.71	0.49 to 1.02
Work carried out[a]			
Outdoors	1.00	1.25	0.33 to 4.65
Indoors	1.00	Reference	Reference
Work in a hot environment†			
Regular/frequently	1.00	0.81	0.24 to 2.70
Irregularly	1.00	Reference	Reference
Shade availability†			
No	1.00	0.58	0.07 to 4.75
Yes or inside	1.00	Reference	Reference
Duration of breaks†			
≤ 10 minutes	1.00	1.27	0.47 to 3.42
>10 minutes	1.00	Reference	Reference
Physical effort at work‡			
Moderate/hard	1.00	1.27	0.43 to 3.77
Slight	1.00	Reference	Reference
Heat/dehydration symptoms†			
Yes	1.00	0.81	0.28 to 2.29
No	1.00	Reference	Reference
Dysuria †			
Yes	1.00	0.42	0.11 to 1.52
No	1.00	Reference	Reference
Fever†			
Yes	1.00	5.77	2.03 to 16.33
No	1.00	Reference	Reference

*Abbreviations: OR: odds ratio; Agricultural work includes all non-sugarcane agricultural work. EIP: economically inactive population. *Probability weighted according to results of growth mixture model; †over the last 6 months; ‡over the last week.*

Supplementary table 6: Age- and education level-adjusted associations* of baseline kidney dysfunction with baseline exposures in male study participants

Characteristic	Preserved and stable eGFR	Baseline dysfunction	
	Reference	OR	95% CI
Alcohol consumption			
Any	1.00	0.85	0.36 to 1.99
None	1.00	Reference	Reference
NSAID use			
Daily/regularly	1.00	2.00	0.65 to 6.10
Never/occasionally	1.00	Reference	Reference
Water sources			
Piped water	1.00	0.74	0.32 to 1.74
Dug well/drilled well	1.00	Reference	Reference
Water hardness			
Softly/moderately hard	1.00	2.13	0.86 to 5.29
Hard/very hard	1.00	Reference	Reference
Total liquid intake in the last 24 hrs			
>5.0 litres/day	1.00	1.18	0.49 to 2.83
≤ 5.0 litres/day	1.00	Reference	Reference
Current occupation			
Sugarcane	1.00	1.82	0.46 to 7.20
Agricultural work	1.00	2.26	0.81 to 6.32
Other occupations/EIP	1.00	Reference	Reference
Main sugarcane role (if ever worked in sugarcane)			
Cane/seed cutter	1.00	3.16	0.69 to 14.47
Seeder	1.00	1.36	0.21 to 8.79
Other cane jobs	1.00	0.59	0.05 to 7.01
Never worked in sugarcane	1.00	Reference	Reference
Current or historical banana work			
Yes	1.00	9.40	3.79 to 23.30
No	1.00	Reference	Reference
Years in sugarcane	1.00	1.03	0.90 to 1.18
Years in agriculture	1.00	1.02	0.93 to 1.11
Work carried out†			
Outdoors	1.00	1.39	0.51 to 3.78
Indoors	1.00	Reference	Reference
Work in a hot environment†			
Regular/frequently	1.00	1.03	0.42 to 2.55
Irregularly	1.00	Reference	Reference
Shade availability†			
No	1.00	0.79	0.27 to 2.23
Yes or inside	1.00	Reference	Reference
Duration of breaks†			
≤ 10 minutes	1.00	2.36	1.01 to 5.55
>10 minutes	1.00	Reference	Reference
Physical effort at work‡			
Moderate/hard	1.00	1.00	0.43 to 2.33
None/slight	1.00	Reference	Reference
Agrochemicals†,§			
Yes	1.00	0.61	0.26 to 1.45
No	1.00	Reference	Reference
Heat/dehydration symptoms†			
Yes	1.00	1.22	0.47 to 3.12
No	1.00	Reference	Reference
Dysuria†			
Yes	1.00	0.33	0.09 to 1.17
No	1.00	Reference	Reference
Fever†			
Yes	1.00	2.58	0.88 to 7.54
No	1.00	Reference	Reference

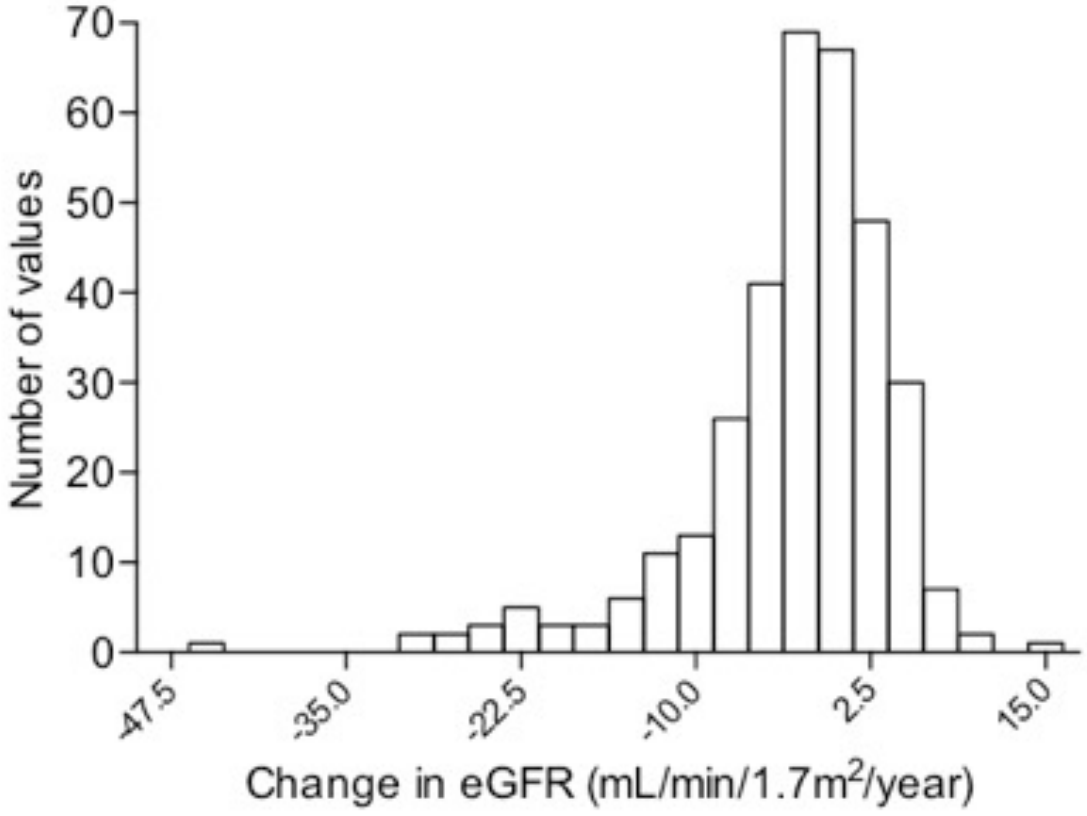
*Abbreviations: OR: odds ratio; NSAID: non-steroidal anti-inflammatory drug; UTI: urinary tract infection; EIP: economically inactive population. Agricultural work includes all non-sugarcane agricultural work. *Probability weighted according to results of growth mixture model; †over the last 6 months; ‡over the last week; §data were collected at the second visit, and include glyphosate, cypermethrin, paraquat and methomyl.*

Supplementary table 7: Age- and education level-adjusted multivariate analysis of associations of baseline exposures with changes in eGFR over the follow-up period in the male population*

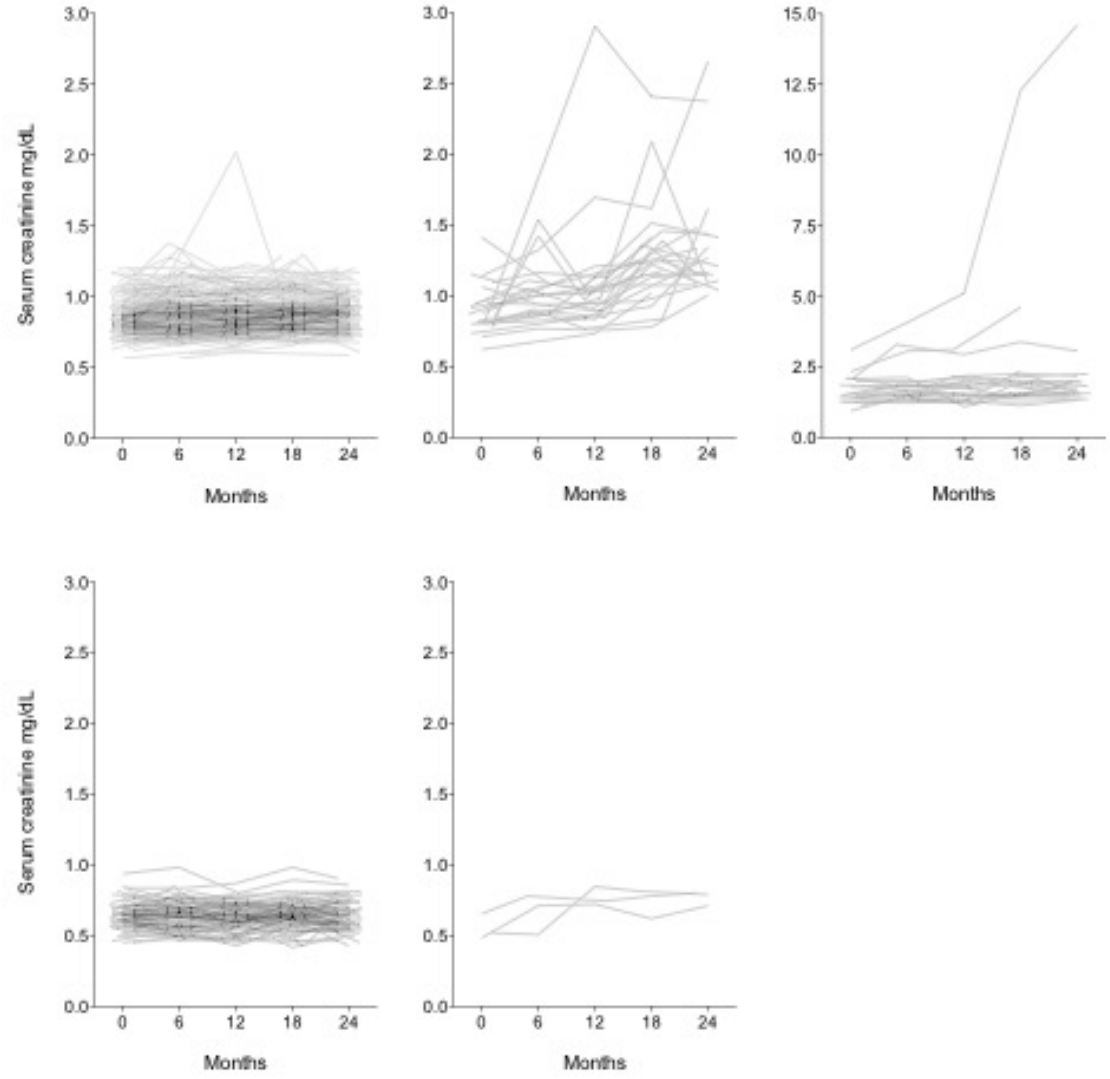
Characteristic	n	Difference in rate of change in $eGFR_{Scr\text{-}Scys}$ (mL/min/1.73 m^2/year)	95% confidence interval
Alcohol consumption			
Any	141	-0.19	-2·14 to 1·75
None	122	Reference [-1·99]	Reference
NSAID use			
Daily/regularly	29	-1.04	-4·13 to 2·04
Never/occasionally	234	Reference [-1·96]	Reference
Water sources			
Piped water	139	-0.71	-2·65 to 1·23
Dug well/drilled well	124	Reference [-1·69]	Reference
Water hardness			
Softly/moderately hard	67	-0.37	-2·58 to 1·82
Hard/very hard	196	Reference [-1·95]	Reference
Total liquid intake in the last 24 hrs			
>5.0 litres/day	85	-0.33	-2·42 to 1·74
≤ 5.0 litres/day	178	Reference [-1·97]	Reference
Current occupation			
Sugarcane	45	-0.28	-3·23 to 2·67
Agricultural work	131	-2.51	-4·65 to -0·37
Other occupations/EIP	87	Reference [-0·67]	Reference
Main sugarcane role (if ever worked in sugarcane)			
Cane/seed cutter	137	-0.25	-2·92 to 2·41
Seeder	47	-1.18	-4·44 to 2·07
Other cane jobs	33	0.04	-3·51 to 3·59
Never worked in sugarcane	46	Reference [-1·81]	Reference
Current or historical banana work			
Yes	47	0.90	-1·57 to 3·39
No	216	Reference [-2·46]	Reference
Years in sugarcane	--	-1.10	-2.03 to -0.17
Years in agriculture	--	0.11	-0.47 to 0.69
Work carried out†			
Outdoors	194	-3.08	-5·23 to -0·92
Indoors	69	Reference [0·19]	Reference
Work in a hot environment†			
Regular/frequently	171	1.17	-0·84 to 3·18
Irregularly	92	Reference [-2·82]	Reference
Shade availability†			
No	73	-3.70	-5·79 to -1·61
Yes or inside	190	Reference [-1·01]	Reference
Duration of breaks†			
≤ 10 minutes	86	-2.16	-4·22 to -0·09
>10 minutes	177	Reference [-1·37]	Reference
Physical effort at work‡			
Moderate/hard	149	-1.31	-3·25 to 0·62
None/slight	114	Reference [-1·35]	Reference
Agrochemicals†,§			
Yes	135	-1.26	-3·20 to 0·67
No	128	Reference [-1·45]	Reference
Heat/dehydration symptoms†			
Yes	175	-0.69	-2·75 to 1·35
No	88	Reference [-1·62]	Reference
Dysuria †			
Yes	72	-0.26	-2·45 to 1·92
No	191	Reference [-1·99]	Reference
Fever†			
Yes	32	-1.74	-4·71 to 1·22
No	231	Reference [-1·89]	Reference

*Abbreviations: NSAID: non-steroidal anti-inflammatory drug; UTI: urinary tract infection; EIP: economically inactive population. Agricultural work includes all non-sugarcane agricultural work. *Coefficients estimated from the eGFR*time interaction. The model displayed a poor fit and the data are provided for information purposes only. †Over the last 6 months; ‡over the last week; §data were collected at the second visit, and include glyphosate, cypermethrin, paraquat and methomyl.*

5. Supplementary Figures



Supplementary figure 1: Distribution of eGFR trajectories in the study population. The decrease in eGFR was calculated by the ordinary least squares method. The sub-group of individuals with a rapid decrease in eGFR represents a small but distinct group of values centring at -20 mL/min/1.73 m^2/year



Supplementary figure 2: Serum creatinine levels in the different eGFR trajectory groups over time

Chapter 5. Identification of young adults at risk of an accelerated loss of kidney function in an area affected by Mesoamerican nephropathy

5.1 Introduction to paper IV

This manuscript was published in BioMedical Central Nephrology (BMC Nephrology). This paper presents a case-control study nested in a cohort study to determine if repeated routine creatinine tests combined with baseline urinary measurements of NGAL levels and can identify the sub-group of individuals at risk of a future rapid decline in kidney function. The study population comprised the three sub-groups of kidney function trajectories for males (213 participants with a preserved/normal eGFR, 25 subjects with rapid decline in kidney function and 25 individuals with established renal dysfunction at baseline).

The outcome was the predictive score to identify individuals susceptible decline in kidney function. Methods used to identify the eGFR trajectories and kidney biomarkers are described in Chapter 4. Initially, the eGFR at the first visit was included in the model (area under the curve: 0.51); addition of uNGAL levels at visit 1 did improve the model, but the model improvement was insufficient to discriminate early kidney damage in this population (area under the curve: 0.75). Based on this finding, other eGFR levels at six and twelve months after baseline were included to improve the final model (area under the curve: 0.89).

The results show that inclusion of uNGAL did not improve the models with eGFR to identify those people at risk of decline in kidney function. However, a question arises of whether other markers such as uric acid, heat shock protein 72 (HSP 72), and interleukin-6 can be more predictive for early kidney damage. In summary, it is urgent to identify a novel kidney marker that could predict a decline in kidney function.

All outputs of each predictive models were included in this paper as supplementary materials in section 5.5.

5.2 Research paper cover sheet

RESEARCH PAPER COVER SHEET

PLEASE NOTE THAT A COVER SHEET MUST BE COMPLETED <u>FOR EACH</u> RESEARCH PAPER INCLUDED IN A THESIS.

SECTION A – Student Details

Student	Marvin Gonzalez-Quiroz
Principal Supervisor	Dorothea Nitsch
Thesis Title	Occupational kidney disease among young populations in northwest Nicaragua

<u>If the Research Paper has previously been published please complete Section B, if not please move to Section C</u>

<u>SECTION B – Paper already published</u>

Where was the work published?	BioMedical Medical Nephrology (BMC Nephrology)		
When was the work published?	2019		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	No		
Have you retained the copyright for the work?*	**Yes**	Was the work subject to academic peer review?	**Yes**

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<u>SECTION C – Prepared for publication, but not to date published</u>

Where is the work intended to be published?	
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Stage of publication	

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I developed the study design with advice and supervision from Dorothea Nitsch, Ben Caplin and Neil Pearce. This study used the dataset from our community-based cohort study that is ongoing in Nicaragua. I conducted the data analysis and drafted the manuscript according to the advice of my supervisors, and this draft manuscript received comments from and was approved by all co-authors. The article was peer reviewed, and my co-authors and I edited the draft in response to reviewers comments.

Student Signature: [redacted] **Date:** January 16/2019

Supervisor Signature: [redacted] **Date:** January 16/2019

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RESEARCH ARTICLE Open Access

Identification of young adults at risk of an accelerated loss of kidney function in an area affected by Mesoamerican nephropathy

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Abstract

Background: After two-years of follow-up of 263 apparently healthy 18- to 30-year-old men in communities affected by Mesoamerican nephropathy (MeN), we identified three distinct case groups: a subgroup with (i) established renal dysfunction (case-group 1); individuals with (ii) a rapid decline in kidney function (case-group 2); and individuals with (iii) stable kidney function (non-cases). This paper investigates whether local tests are potentially useful for the timely identification of these case groups.

Methods: Creatinine levels were measured in local laboratories every six months for two years. Aliquots were sent to a centralized laboratory for measurements of cystatin C and creatinine levels. We investigated agreement between the locally and centrally measured creatinine-based Chronic Kidney disease Epidemiology Collaboration (CKD-EPI) equation for estimating the Glomerular Filtration Rate (eGFR). A logistic regression analysis was used to assess predictive factors for case groups 1 and 2 compared to non-cases. Predictive variables were locally measured eGFR, and urinary neutrophil gelatinase-associated lipocalin (uNGAL) levels. The discrimination performance of the model was assessed using the area under the receiver operating characteristic curve (AUC).

Results: Considerable variation in local eGFR measurements was observed. The prediction model for case-group 1 included baseline kidney function and with or without uNGAL (AUC = 0.98, 95% confidence interval (CI), 0.91–1.00). The prediction model for case-group 2 also required $eGFR_{Scr}$ at six and twelve months after baseline, with or without uNGAL levels (AUC = 0.88; 95% CI 0.80–0.99).

Conclusions: Established renal dysfunction was detected at a single time point using local measurements and uNGAL. For the detection of a rapid decline in kidney function over time, at least 2 more measurements at six and twelve months are needed.

Keywords: Mesoamerican nephropathy, Chronic kidney disease of unknown aetiology, Prediction, Kidney function status, Serum creatinine, uNGAL, ROC, Nicaragua

Background

Mesoamerican nephropathy (MeN) is a major public health and economic problem affecting rural and agricultural communities in Mesoamerica. Over the last decade, MeN, also known as chronic kidney disease of unknown aetiology (CKDu), has caused the deaths of thousands of vulnerable young male agricultural workers, particularly sugarcane workers and other workers (agricultural and non-agricultural) who work in extremely hot conditions along the Pacific coast of Mesoamerica [1–5]. MeN has devastating consequences for patients, family members, communities and the country, with patients who are diagnosed with the disease progressing to end stage renal disease as young and middle-aged adults. Renal replacement therapy options are expensive and limited in Mesoamerica, resulting in the high mortality of patients with MeN [6, 7].

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This original article has been read and approved by all authors listed above and is not under consideration for publication elsewhere.
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MeN disease has some unique characteristics, including the absence of traditional risk factors (hypertension and diabetes) [1, 8, 9]. A recent systematic review identified that the male gender, living in lowlands, a family history of CKD and a high water-intake are associated with CKDu [10]. Some studies have reported elevated urinary neutrophil gelatinase-associated lipocalin (uNGAL) and N-acetyl-β-D-glucosaminidase (NAG) levels in sugarcane workers [11, 12]. Therefore, one current hypothesis is that MeN is caused by repetitive acute kidney injury due to multiple risk factors, such as working conditions and heat [1, 8].

We have recently conducted a community-based cohort study in the affected region. We used established biomarkers of kidney function (serum cystatin C (Scys) and serum creatinine (Scr) levels) that were measured in stored samples from baseline and 6-month follow-up visits in a central laboratory at the end of the study (five measures in total) to determine the decline in kidney function after 2 years of follow-up. [13, 14] Although we attempted to recruit people without established kidney disease at baseline, 25 people (10.5% of the cohort) who were apparently healthy but in fact had established kidney dysfunction at baseline were included. An additional sub-group of males (10.5% of the total cohort) exhibited an extremely rapid decline in kidney function of 18.2 mL/min/1.73 m^2/year from normal levels [14]. The observed dramatic loss of renal function among a sizeable sub-group of an apparently healthy young population is a concern.

The local industries in Nicaragua have established baseline screening programmes for kidney dysfunction using a single local creatinine test [15]. Despite the use of this local screening programme, a sub-group of people with established kidney disease was recruited in our study; therefore, a question arises of whether other information is needed in addition to a single serum creatinine measurement to detect MeN. In addition, the identification of the subgroup that will experience a rapid decline in kidney function will be beneficial to provide advice (e.g., avoidance of known nephrotoxins, such as non-steroidal anti-inflammatory drugs) and potentially to implement interventions (e.g., improve work conditions, clean water etc., if these interventions are proven to be effective) to prevent or delay future kidney function loss. Serial measurements of serum creatinine levels over more than 2 years are not the usual practice in Nicaragua. Therefore, the purpose of this analysis is to determine if repeated local creatinine tests combined with urinary measurements of uNGAL levels can identify the subgroup of individuals at risk of a future rapid decline in kidney function.

Methods

Study population

These analyses are based on our existing community-based follow-up study of a decline in kidney function among affected communities in Nicaragua. The rationale and study design have been published elsewhere [13]. Briefly, we recruited 350 apparently healthy young adults aged 18–30 years without a known diagnosis of CKDu and traditional risk factors, and followed them for two years. All eligible healthy males and a sample of females from 9 communities in northwest Nicaragua were enrolled in our study. Biological samples, anthropometric measurements and questionnaire data were collected at baseline and then at six-month intervals. The outcome was the kidney function status, and we classified the participants into three categories: (i) established renal dysfunction; (ii) a rapid decline in kidney function; and (iii) stable kidney function [14].

Clinical measurements

Gold standard

All samples were analysed in a single batch to reduce time-dependent measurement errors in the assays after two years of follow-up. At the end of the follow-up period, stored (− 80 °C) serum aliquots from all study visits were transferred to the Clinical Trial Service Unit at Oxford University. Scr levels were quantified using a Beckman AU680 Chemistry Analyser (Jaffe compensated method) and calibrated against the IDMS-traceable creatinine standard. Scys levels were measured using Siemens BN ProSpec (nephelometry) [14]. The CKD-EPI equation for serum creatinine and cystatin c levels was used to estimate the estimated glomerular filtration rate, or eGFR ($eGFR_{Scr\text{-}Scys}$) [16].

Routine local measurements

Serum creatinine (Scr) levels were also measured locally (in the Biochemistry Department at the National Autonomous University of Nicaragua-Leon) using a ChemWell® 2910 (Awareness Technology, EEUU) auto analyser (Jaffe compensated method) [17, 18]. Local Scr values were multiplied by 0.95, as they were not calibrated to an IDMS-traceable creatinine standard [19]. Kidney function was calculated using the estimated glomerular filtration rate according to the CKD-EPI formula by determining Scr levels [16] at each study visit.

Urine markers

Creatinine and albumin levels in baseline urine samples, which were previously frozen and stored at − 80 °C for 2.5 years, were measured using the Jaffe and bromocresol green reactions (Sigma-Aldrich, MAK124), respectively. Samples were read at a specific wavelength using the Biochrom EZ Read 400 Microplate Reader. Urinary neutrophil gelatinase-associated lipocalin (uNGAL) levels were measured in a subsample. Samples from fifty-five randomly selected participants with stable kidney function and samples from all participants in the other two subgroups (rapid decline in kidney function

and patients with established renal dysfunction; both of which are defined below) were analysed. uNGAL levels were measured using enzyme-linked immunoassay (ELH-Lipocalin2, RayBiotech), according to the manufacturer's instructions. Albumin and uNGAL levels are reported as ratios to urinary creatinine levels measured using the Jaffe method (Sigma-Aldrich, C4255) [14].

Definitions of outcome categories

A growth mixture model (GMM) was used to identify three sub-groups of kidney function in males using the gold standard eGFR after 2 years, as previously described [14]. A group with established renal dysfunction at baseline (mean $eGFR_{Scr\text{-}Scys}$: 58 mL/min/1.73 m^2) was investigated, which for the purpose of this paper, is defined as case-group 1. Then, a group with normal baseline kidney function (mean eGFR: 112 mL/min/1.73 m^2) showed a rapid decline in $eGFR_{Scr\text{-}Scys}$ of − 18.2 mL/min/1.73 m^2/year and were designated case-group 2, i.e., rapid decliners. The remaining study participants were 'non-cases' with stable kidney function, i.e., men with persistently stable kidney function (mean baseline eGFR: 116 mL/min/1.73 m^2) and an annual decline in $eGFR_{Scr\text{-}Scys}$ of only − 0.6 mL/min/1.73 m^2/year over two years. The analyses compared each of the two case groups with the non-cases. Given the small number of affected females exhibiting a rapid decline in kidney function (3 cases), formal analyses were restricted to males.

Variables

Each participant completed a baseline questionnaire by face-to-face interview that included demographic data, current and previous occupations, lifestyle factors, medications, liquid intake, and dehydration symptoms [13, 14]. The season was defined as summer and winter. Work performed was classified as outdoor and indoor work. For this analysis, we used the eGFR and uNGAL levels, but in a sensitivity analysis the baseline questionnaire data (age, season, work performed, urinary albumin-creatinine ratio (UACR)) were included as part of the full model. UACR was categorized as a ratio ≥ 30 mg/g or < 30 mg/g [20].

Statistical analysis

Descriptive analyses of continuous variables (age, serum creatinine, $eGFR_{Scr}$, $eGFR_{Scr\text{-}Scys}$, and uNGAL) stratified by case/non-case status were conducted, and are reported as the means (SD) or medians and interquartile ranges (IQRs). Categorical variables (season, work performed and UACR) are reported as frequencies and percentages.

For the identification of cases of MeN in the local setting, local measurements are required; therefore, the current analysis is based on the routine creatinine measurements performed after each visit. The agreement between the estimated GFR based on the Scr Jaffe assay conducted at the laboratory in Nicaragua and the $eGFR_{Scr}$ from Oxford (gold-standard measure) was evaluated at three time points (baseline, 6 and 12 months) using a Bland-Altman plot.

For case-group 1, we first assessed whether the routine eGFR data derived from local creatinine measurements identified people with established kidney damage. Logistic regression models were also performed to compare case-group 1 with non-cases. Model 1 included $eGFR_{Scr}$ at baseline; Model 2 included the Model 1 covariate plus uNGAL levels. Along with variables included in Models 1 and 2, subsequent models for non-cases and case-group 2 also included the 6- and/or 12-month eGFR with or without uNGAL levels.

For all models, receiver operating characteristic (ROC) curves were generated to calculate the area under the ROC curve (AUC). An AUC greater than 0.80 was considered a suitable discrimination performance for the model.

In order to check that the results are not confounded, we repeated the analysis using only the continuous eGFR, and uNGAL measurements with further adjustments for other variables such us age, season, work performed, and urinary albumin-creatinine ratio (UACR) (Table 1).

All statistical analyses were performed using Stata software, version 14 (Stata Corp.).

Results

Agreement between clinical measurements – Routine $eGFR_{Scr\text{-}Nicaragua}$ compared to the gold-standard $eGFR_{Scr\text{-}Oxford}$

In daily clinical practice, $eGFR_{Scr}$ measurements are used, and we compared these values to gold-standard $eGFR_{Scr\text{-}Oxford}$. The mean difference in baseline kidney function based on serum creatinine levels measured in Nicaragua compared to serum creatinine levels measured in Oxford was − 10.47 mL/min/1.73 m^2 (95% CI -11.90 to − 9.03), suggesting that Nicaraguan kidney function was overestimated by local measurements. The limits of agreement ranged from − 37.24 to 16.30 mL/min/1.73 m^2 with a variability of 3.7%, suggesting that local measurements varied. No evidence of the measurement error in $eGFR_{Scr}$ was observed, with a poor correlation between the difference and the sums ($r = -0.06$; $P = 0.213$) (Additional file 1: Figure S1A). The mean difference in $eGFR_{Scr\text{-}Nicaragua}$ after 6 months was − 8.39 mL/min/1.73 m^2 (95% CI -9.91 to − 6.88), with a range of 22.07 to 152.35 mL/min/1.73 m^2. The limits of agreement were − 35.09 to 18.30 mL/min/1.73 m^2 and the variability was 5.4% (Additional file 1: Figure S1B). Finally, the mean difference in $eGFR_{Scr\text{-}Nicaragua}$ at the third study visit was + 3.44 mL/min/1.73 m^2 (95% CI 1.76 to 5.11), with a range of 15.54 to 146.33 mL/min/1.73 m^2. The limits of agreement were − 26.90 to 33.17 mL/min/1.73 m^2 and the variability was 7.2%. A weak correlation between the difference and the sums was observed for both lab results ($r = -0.11$; $P = 0.042$) (Additional file 1: Figure S1C).

Table 1 Variables outlined in each of the models stratified by case-groups

Variables	Case-group 1		Case-group 2							
	Model 1	*Model 2*	*Model 3*	*Model 4*	*Model 5*	*Model 6*	*Model 7*	*Model 8*	*Model 9*	*Model 10*
Age	X	X	X	X	X	X	X	X	X	X
Season	X	X	X	X	X	X	X	X	X	X
Occupation (outdoor work)	X	X	X	X	X	X	X	X	X	X
UACR	X	X	X	X	X	X	X	X	X	X
UNGAL		X		X		X		X		X
$eGFR_{Scr}$ at baseline	X	X	X	X	X	X	X	X	X	X
$eGFR_{Scr}$ at 6 months					X	X			X	X
$eGFR_{Scr}$ at 12 months							X	X	X	X

General characteristics stratified by kidney function trajectories

The mean age at baseline for the entire men population was 23.7 ± 3.8 years. The majority of males (74%) reported that they worked outdoors. Table 2 shows the routine local clinical kidney measurements at baseline stratified according to the observed renal outcome groups after 2 years of follow-up. At baseline, no differences were observed between controls and case-group 2. However, a subgroup of males had a high serum creatinine level and low $eGFR_{Scr}$ at baseline, and were unaware of this condition at recruitment into the study (case--group 1). Case-group 1 displayed a high urinary uNGAL level at baseline. However, the percentages of males displaying a UACR ≥30 mg/g were 4.7% among participants with stable kidney function and 16% among participants with established renal dysfunction (case-group 1).

Model for predicting established renal dysfunction

The prediction score comparing stable kidney function (non-cases) and established renal dysfunction (case-group 1) without uNGAL levels by using a single $eGFR_{Scr}$ measurement was excellent ($P < 0.001$), showing an AUC of 0.97 (95% CI 0.93–1.00) (Additional file 1: Table S1; Model 1). The AUC for Model 2, where a single $eGFR_{Scr}$ measurement and uNGAL levels did not improve the prediction compared with established predictors, was 0.98 (95% CI 0.95–1.00). The c statistic was the same for both models. (Fig. 1 and Additional file 1: Table S1).

Model for predicting a rapid decline in kidney function

The AUC just for the estimated glomerular filtration rate at baseline showed a poor discrimination to identify populations at risk of a rapid decline in kidney function (case-group 2). As shown in Fig. 2a, the discrimination power of the model at baseline was poor, with an AUC of 0.51 (95% CI 0.34–0.69), and when uNGAL was added to the logistic regression model, the AUC was 0.75 (95% CI 0.57–0.92). The c statistic for Model 4 with uNGAL levels was 0.24 higher, indicating a significant improvement compared with Model 3 that did not include uNGAL levels.

Figure 2b shows the results of the model without uNGAL levels, but with an additional measurement of $eGFR_{Scr}$ at 6 months (Model 5). The discrimination power of the model was somewhat improved (compared to Model 3), with an AUC of 0.71 (95% CI 0.56–0.85). For the same variables with the addition of uNGAL levels (Model 6), the AUC was 0.73 (95% CI 0.58–0.88). The c statistic for model 6 (with uNGAL levels) was 0.02.

Figure 2c shows the results of the models including an $eGFR_{Scr}$ recorded 12 months after the baseline measure

Table 2 Baseline characteristics of apparently healthy young men in northwest Nicaragua stratified by trajectories of a future decline in kidney function using gold standard measurements (CKDEPI Creat/Cyst eGFR) ($n = 263$)

Trajectories of the decline in kidney function	N	Age at baseline (Mean; SD)	Serum creatinine level at baseline‡ (Median; IQR)	$eGFR_{Scr}$ at baseline‡ (Median; IQR)	$eGFR_{Scr\text{-}Scys}$ at baseline† (Median; IQR)	Urinary NGAL level at baseline◊ (Median; IQR)	UACR ≥30 mg/dL at baseline◊ (n; %)	Outdoor work at baseline (n; %)
Stable kidney function (–0.6 mL/min/1.73 m^2/year)	213	23.6 ± 3.89	0.76 (0.57–0.76)	131 (124–140)	118 (108–125)	5.04 (4.7–5.4)	10 (4.7)	151 (70.9)
Rapid decline in kidney function (– 18.2 mL/min/1.73 m^2/year)	25	23.3 ± 3.65	0.66 (0.47–0.76)	132 (123–152)	117 (103–124)	5.20 (5.0–5.5)	0 (0)	24 (96.0)
Renal dysfunction (– 3.8 mL/min/1.73 m^2/year)	25	25.4 ± 2.97	1.23 (1.14–1.52)	80 (62–91)	56 (49–68)	5.7 (5.5–5.7)	4 (16.0)	19 (76.0)
Total	263	23.7 ± 3.82	0.76 (0.66–0.85)	129 (121–139)	116 (102–125)	5.20 (4.9–5.7)	14 (5.3)	194 (73.8)

‡Routine local test measurement. †Gold-standard outcome measured in Oxford 2 years later. ◊Urinary biomarkers of kidney injury

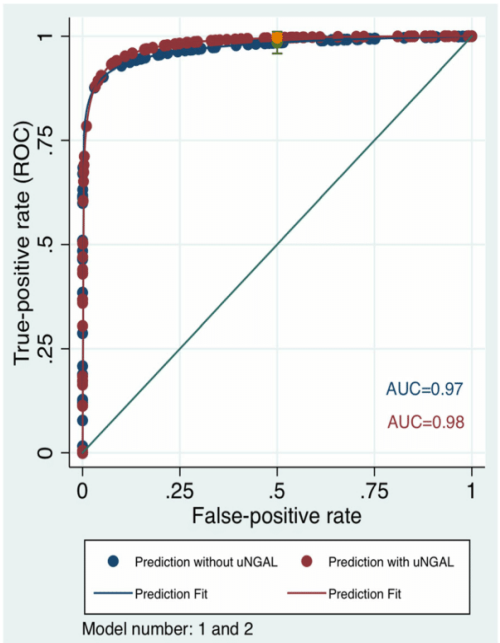

Fig. 1 ROC curves for the model predicting stable kidney function versus established renal dysfunction using eGFR and uNGAL. The 95% confidence intervals for the ROC curves (0.5) are displayed

(excluding the 6-month measurement; Model 7), with an AUC of 0.80 (95% CI 0.70–0.91). The addition of uNGAL levels (Model 8) to this model yielded an AUC of 0.81 (95% CI 0.72–0.91). The c statistic was almost indistinguishable between model 7 and model 8, suggesting that uNGAL levels do not additional predictive value to model 7.

The best prediction of a future rapid decline in kidney function (Fig. 2d) was achieved using 3 $eGFR_{Scr}$ measurements: baseline, 6 months and 12 months (Model 9). This model yielded an AUC of 0.88 (95% CI 0.79–0.98) and an AUC of 0.89 (95% CI 0.80–0.99) when urinary uNGAL levels were incorporated into the model (Model 10). Again, the c statistics were similar between Models 9 and 10 (Fig. 2 and Additional file 1: Table S2).

Results of the sensitivity analysis using eGFR, uNGAL measurements plus baseline questionnaire data (age, season, work performed, and UACR) were not appreciably changed when compared to the main analysis (Additional file 1: Figures S2 and S3 and Additional file 1: Table S3 and S4).

Discussion

Using a gold-standard measurement of decline in kidney function, we classified the participants into three categories: (i) established renal dysfunction (case-group 1); (ii) a rapid decline in kidney function (case-group 2); and (iii) stable kidney function (non-cases). We then compared each of the two case-groups with the non-cases. While it was straightforward to identify individuals with already established renal dysfunction at baseline using routine laboratory data together with uNGAL (AUC: 0.98; 95% CI: 0.95 to 1.00), a follow-up for at least one year with 6 monthly measurements was required to identify most people who will suffer from a future rapid decline in kidney function from normal participants (AUC: 0.89; 95% CI: 0.80 to 0.99 and a false positive rate of 0.5). We did not observe a difference in the ROC values in the final model with and without uNGAL levels and note that the confidence intervals nearly overlapped.

Good quality laboratory results are very important for an accurate diagnosis and clinical decision making [21–24]. The laboratory in Nicaragua is not accredited to ISO standards; however, as happens in routine clinical care, the machines are regularly recalibrated. Routine clinical care procedure do not standardly bank blood samples for several years to then retrospectively assess decline in kidney function. We showed that routine clinical care measurements displayed considerable time-dependent variability. The variability in the local Scr measurements increased the imprecision between the $eGFR_{Scr\text{-}Nicaragua}$ and the gold-standard ($eGFR_{Scr\text{-}Oxford}$) of 3.7% at baseline and 7.2% at the third study visit. However, for the eGFR determinations performed following the baseline visit, measurement error appeared to have decreased as the laboratory obtaining a better machine recalibration, maintenance and the implementation of external quality control standards. Based on these findings, health care providers and researchers must be aware of the challenges of using local serum creatinine measurements.

To date, many prediction models have been developed and validated for determining the progression of kidney disease to ESRD [25, 26]. Roy et al. developed a prediction model and showed how a novel biomarker (uNGAL) improves prediction of CKD progression in the CRIC study [27]. Their model included demographic data plus uNGAL levels, and the model without uNGAL levels showed a poor discrimination power (AUC = 0.69), whereas the model with uNGAL levels exhibited good discrimination (AUC = 0.82). Our model with uNGAL levels used to distinguish participants with renal dysfunction (case-group 1) did not add predictive value beyond the established predictors (AUC = 0.98) compared to the model without uNGAL levels (AUC = 0.97).

When predicting a future rapid decline in kidney function (case-group 2), the addition of uNGAL levels to the model that included a single $eGFR_{Scr}$ measurement increased the AUC from 0.51 to 0.75 (Model 3 vs Model 4). However, in the models including three eGFR measurements (Model 9 vs Model 10) the two curves were almost indistinguishable (e.g., AUCs with and without uNGAL levels were 0.88 and 0.89 respectively for models 9 and 10). Thus, no discernible

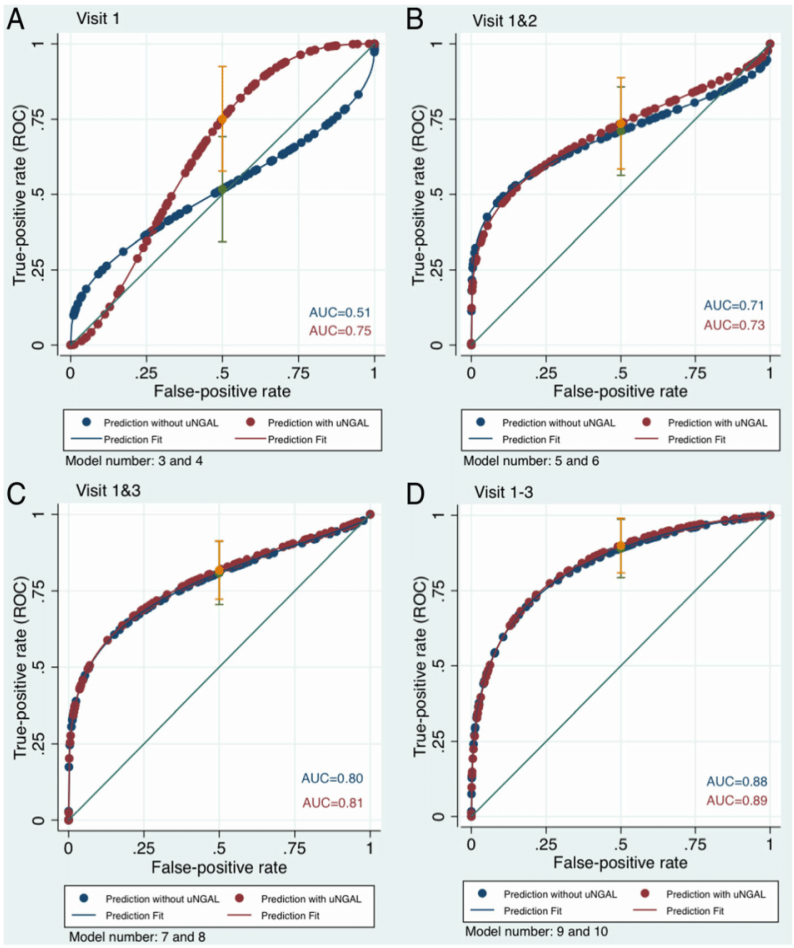

Fig. 2 ROC curves for the model predicting stable kidney function versus a rapid decline in kidney function using eGFR and uNGAL. The 95% confidence intervals for ROC curves (0.5) are displayed. The blue label means: Area under the curve without uNGAL. The maroon label means: Area under the curve with uNGAL

improvement in prediction was observed when uNGAL levels were added to a model containing more than one creatinine measurement recorded over time.

The key implication from our study for future cohort and/or intervention studies of CKDu is that the minimal follow-up period is one year, and 6 monthly measurements are required to distinguish the progressive loss of eGFR from stable kidney function. Any study with a shorter follow-up period less likely to detect significant progression among participants with normal function at baseline.

Our study has methodological strengths. First, this study is derived from a community-based longitudinal study of apparently healthy young adults in high-risk areas for CKDu. Second, the variables that were used to calculate the prediction score are easy to obtain at any health level (i.e., primary, secondary and tertiary health levels) and can be applied by doctors or any health professional.

Our study has some limitations that should be noted. External validation was not performed because this community-based cohort study is the first in the region, and no other comparable cohorts are available. Second, we were unable to assess the effect of the season in which eGFR measures are performed because almost all participants were recruited before the harvest season [14].

Conclusions

In conclusion, established renal dysfunction was detected at a single time point using local measurements of eGFR and uNGAL. However, the detection of a rapid decline in kidney function over time requires at least 2 measurements, ideally at least twelve months apart. In addition, local routine clinical measurements of creatinine levels are affected by time-dependent measurement error.

Additional file

Additional file 1: Figure S1. Bland-Altman plot of eGFR based on serum creatinine levels measured in Nicaragua and serum creatinine levels measured in London. **Figure S2.** ROC curves for the model predicting stable kidney function versus established renal dysfunction. The 95% confidence intervals for the ROC curves (0.5) are displayed. **Figure S3.** ROC curves for the model predicting stable kidney function versus a rapid decline in kidney function. The 95% confidence intervals for ROC curves (0.5) are displayed. **Table S1.** Multivariate adjusted logistic regression analysis for eGFR and uNGAL with established renal dysfunction at baseline among apparently healthy young males. **Table S2.** Multivariate adjusted logistic regression analysis for eGFR and uNGAL with a rapid decline in kidney function at baseline among apparently healthy young males. **Table S3.** Multivariate adjusted logistic regression analysis of factors associated with established renal dysfunction at baseline among apparently healthy young males. **Table S4.** Multivariate adjusted logistic regression analysis for factors associated with a rapid decline in kidney function at baseline among apparently healthy young males. (PDF 600 kb)

Abbreviations

AUC: Area under the receiver operating characteristic curve; CI: Confidence interval; CISTA: Centro de Investigación en Salud, Trabajo y Ambiente; CKD: Chronic kidney disease; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; CKDu: Chronic kidney disease of unknown aetiology; CRIC: Chronic renal insufficiency cohort; eGFR: Estimated glomerular filtration rate; ESRD: End-stage renal disease; GMM: Growth mixture model; IDMS: Isotope dilution mass spectrometry; IQR: Interquartile range; MeN: Mesoamerican nephropathy; NAG: N-acetyl-β-D-glucosaminidase; ROC: Receiver operating characteristic; Scr: Serum creatinine; Scys: Serum cystatin C; SD: Standard deviation; UACR: Urinary albumin-creatinine ratio; UK: United Kingdom; uNGAL: Urinary neutrophil gelatinase-associated lipocalin

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Availability of data and materials

The dataset analysed in the current study is available from the corresponding author.

Authors' contributions

This study was designed by MG, NP, BC and DN. Data were collected by MG, ES, NP and BC. Biological samples were analysed by ES. The data were analysed and interpreted by MG, ES, NP, BC and DN. All authors read and approved the final draft of the manuscript.

Ethics approval and consent to participate

The study was approved by the Institutional Review Boards at the Medical Faculty of UNAN-León (Ref: FWA00004523/IRB00003342) and the Research Ethics Committee of the London School of Hygiene and Tropical Medicine (Ref: 8643/14363) in 2014. A written consent form was provided by all participants in the follow-up study, in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors have no competing interests to declare.

Publisher's Note

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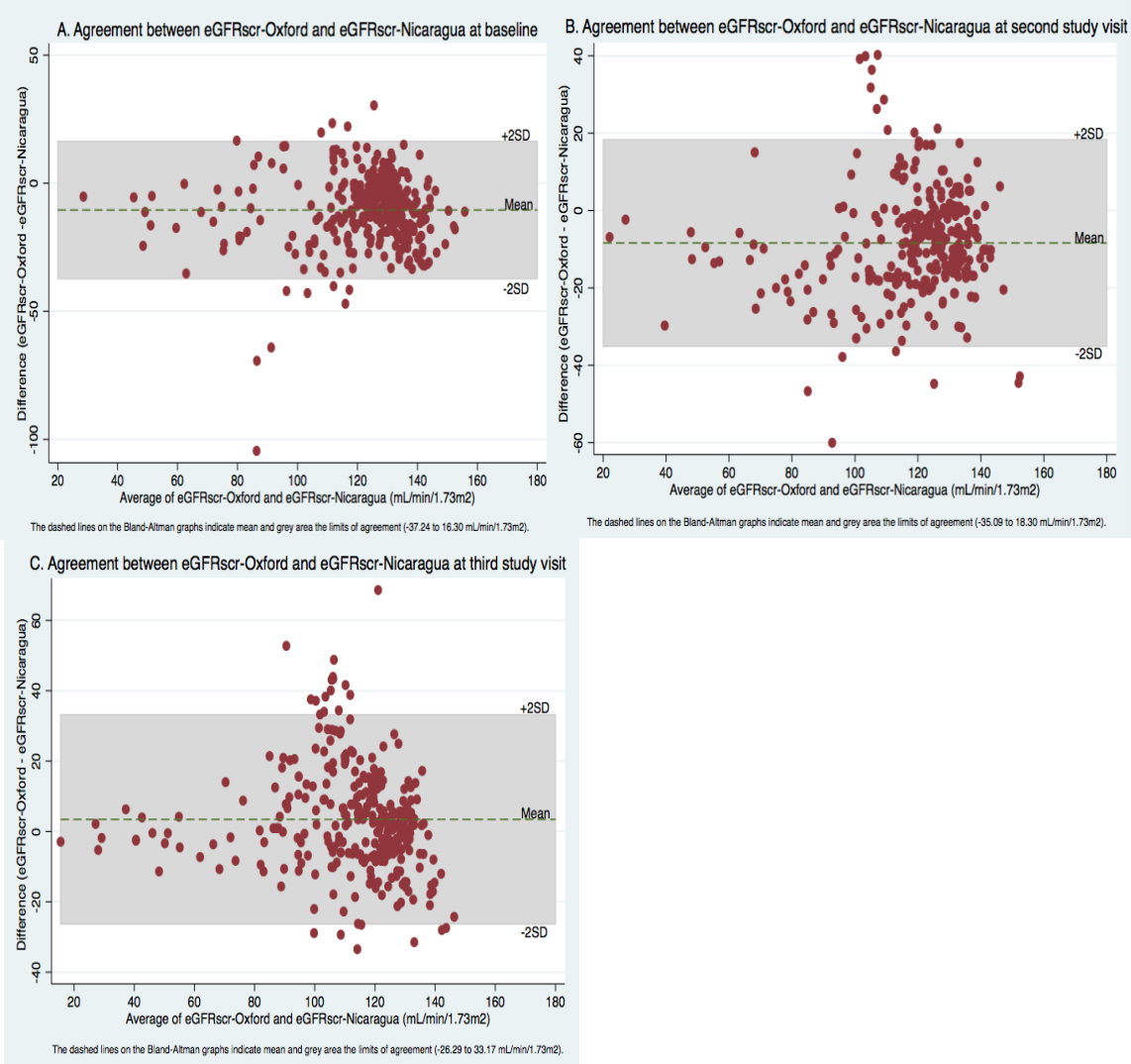
References

1. Wegman D, Crowe J, Hogstedt C, Jakobsson K, Wesseling C, editors: Mesoamerican nephropathy: report from the second international research workshop on MeN. In: *SALTRA*. Edited by Central American Institute for Studies on Toxic Substances (IRET-UNA) and Program on Work EaHiCAS, vol. 33. Heredia, C.R: SALTRA/IRET-UNA; 2016.
2. Wesseling C, Aragon A, Gonzalez M, Weiss I, Glaser J, Rivard C, Roncal-Jimenez C, Correa-Rotter R, Johnson RJ. Heat stress, hydration and uric acid: a cross-sectional study in workers of three occupations in a hotspot of mesoamerican nephropathy in Nicaragua. BMJ Open. 2016;6(12):1–12.
3. Ordunez P, Saenz C, Martinez R, Chapman E, Reveiz L, Becerra F. The epidemic of chronic kidney disease in Central America. Lancet Glob Health. 2014;2(8):e440–1.
4. Correa-Rotter R, Wesseling C, Johnson RJ. CKD of unknown origin in Central America: the case for a Mesoamerican nephropathy. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2014;63(3):506–20.
5. Weiner DE, McClean MD, Kaufman JS, Brooks DR. The central American epidemic of CKD. Clin J Am Soc Nephrol. 2013;8(3):504–11.
6. Weaver VM, Fadrowski JJ, Jaar BG. Global dimensions of chronic kidney disease of unknown etiology (CKDu): a modern era environmental and/or occupational nephropathy? BMC Nephrol. 2015;16:145.
7. Garcia-Trabanino R, Trujillo Z, Colorado AV, Magana Mercado S, Henriquez CA. En nombre de la Asociacion de Nefrologia e Hipertension arterial de El S: prevalence of patients receiving renal replacement therapy in El Salvador in 2014. Nefrologia : publicacion oficial de la Sociedad Espanola Nefrologia. 2016;36(6):631–6.
8. Wesseling C, Crowe J, Hogstedt C, Jakobsson K, Lucas R, Wegman D, editors: First international research workshop on mesoamerican nephropathy (MeN). In: *SALTRA*. Edited by Central American Institute for Studies on Toxic Substances (IRET-UNA) and Program on Work EaHiCAS. Heredia, C.R.: SALTRA/IRET-UNA; 2013.
9. Torres C, Aragon A, Gonzalez M, Lopez I, Jakobsson K, Elinder CG, Lundberg I, Wesseling C. Decreased kidney function of unknown cause in Nicaragua: a community-based survey. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2010;55(3):485–96.
10. Gonzalez-Quiroz M, Pearce N, Caplin B, Nitsch D. What do epidemiological studies tell us about chronic kidney disease of undetermined cause in Mesoamerica?: a systematic review and meta-analysis. Clin Kidney J. 2017:1–11.
11. Laws RL, Brooks DR, Amador JJ, Weiner DE, Kaufman JS, Ramirez-Rubio O, Riefkohl A, Scammell MK, Lopez-Pilarte D, Sanchez JM, et al. Biomarkers of kidney injury among Nicaraguan sugarcane workers. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2016;67(2):209–17.
12. Wesseling C, Aragon A, Gonzalez M, Weiss I, Glaser J, Bobadilla NA, Roncal-Jimenez C, Correa-Rotter R, Johnson RJ, Barregard L. Kidney function in sugarcane cutters in Nicaragua - a longitudinal study of workers at risk of Mesoamerican nephropathy. Environ Res. 2016;147:125–32.
13. Gonzalez-Quiroz M, Camacho A, Faber D, Aragon A, Wesseling C, Glaser J, Le Blond J, Smeeth L, Nitsch D, Pearce N, et al. Rationale, description and baseline findings of a community-based prospective cohort study of kidney function amongst the young rural population of Northwest Nicaragua. BMC Nephrol. 2017;18(1):16.
14. Gonzalez-Quiroz M, Smpokou ET, Silverwood RJ, Camacho A, Faber D, Garcia BR, Oomatia A, Hill M, Glaser J, Le Blond J, et al. Decline in kidney

function among apparently healthy young adults at risk of Mesoamerican nephropathy. J Am Soc Nephrol. 2018;29:2200–12.
15. Laws RL, Brooks DR, Amador JJ, Weiner DE, Kaufman JS, Ramirez-Rubio O, Riefkohl A, Scammell MK, Lopez-Pilarte D, Sanchez JM, et al. Changes in kidney function among Nicaraguan sugarcane workers. Int J Occup Environ Health. 2015;21(3):241–50.
16. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, Kusek JW, Manzi J, Van Lente F, Zhang YL, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med. 2012;367(1):20–9.
17. Wyss M, Kaddurah-Daouk R. Creatine and creatinine metabolism. Physiol Rev. 2000;80(3):1107–213.
18. Diagnostics R: Creatinine Jaffe G E N 2, Compensated Method for serum and Plasma. Mannheim: Roche Diagnostics. In. Edited by Diagnostics R: Roche Diagnostics; 2006.
19. Levey AS, Coresh J, Greene T, Marsh J, Stevens LA, Kusek JW, Van Lente F. Chronic kidney disease epidemiology C: expressing the modification of diet in renal disease study equation for estimating glomerular filtration rate with standardized serum creatinine values. Clin Chem. 2007;53(4):766–72.
20. KDIGO. KDIGO 2012 clinical practice guideline for the evaluation and Management of Chronic Kidney Disease. Kidney Int Suppl. 2013;3(1):1–150.
21. Selvin E, Juraschek SP, Eckfeldt J, Levey AS, Inker LA, Coresh J. Within-person variability in kidney measures. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2013;61(5):716–22.
22. Schmidt RL, Straseski JA, Raphael KL, Adams AH, Lehman CM. A risk assessment of the Jaffe vs enzymatic method for creatinine measurement in an outpatient population. PLoS One. 2015;10(11):e0143205.
23. Hoste L, Deiteren K, Pottel H, Callewaert N, Martens F. Routine serum creatinine measurements: how well do we perform? BMC Nephrol. 2015;16:21.
24. Padala S, Tighiouart H, Inker LA, Contreras G, Beck GJ, Lewis J, Steffes M, Rodby RA, Schmid CH, Levey AS. Accuracy of a GFR estimating equation over time in people with a wide range of kidney function. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2012;60(2):217–24.
25. Tangri N, Stevens LA, Griffith J, Tighiouart H, Djurdjev O, Naimark D, Levin A, Levey AS. A predictive model for progression of chronic kidney disease to kidney failure. Jama. 2011;305(15):1553–9.
26. Bang H, Vupputuri S, Shoham DA, Klemmer PJ, Falk RJ, Mazumdar M, Gipson D, Colindres RE, Kshirsagar AV. SCreening for occult REnal disease (SCORED): a simple prediction model for chronic kidney disease. Arch Intern Med. 2007;167(4):374–81.
27. Roy J, Shou H, Xie D, Hsu JY, Yang W, Anderson AH, Landis JR, Jepson C, He J, Liu KD, et al. Statistical methods for cohort studies of CKD: prediction modeling. Clin J Am Soc Nephrol. 2017;12(6):1010–7.

5.5 Supplementary materials

Supplementary figure 1: Bland-Altman plot of eGFR based on serum creatinine levels measured in Nicaragua and serum creatinine levels measured in London



Supplementary table 1: Multivariate adjusted logistic regression analysis for eGFR and uNGAL associated with established renal dysfunction at baseline among apparently healthy young males

Factors	Model 1 (n=80)				Model 2 (n=80)			
	Without uNGAL levels				With uNGAL levels			
	Coefficient	SE	95% CI	P-value	Coefficient	SE	95% CI	P-value
$eGFR_{Scr}$ at baseline	-0.14	0.03	-0.22 to -0.07	<0.001	-0.15	0.04	-0.24 to -0.06	<0.001
Urinary NGAL	--	--	--	--	3.16	1.44	0.34 to 5.99	0.028

Abbreviations: SE: standard error, uNGAL: urinary neutrophil gelatinase-associated lipocalin, $eGFR_{Scr}$: estimated glomerular filtration rate based on locally measured creatinine levels.

Supplementary table 2: Multivariate adjusted logistic regression analysis of eGFR and uNGAL associated with a rapid decline in kidney function at baseline among apparently healthy young males

Factors	Figure 2A								Figure 2B			
	Model 3 without uNGAL levels (n=79)				Model 4 with uNGAL levels (n=79)				Model 5 without uNGAL levels (n=68)			
	Coefficient	SE	95% CI	P-value	Coefficient	SE	95% CI	P-value	Coefficient	SE	95% CI	P-value
$eGFR_{Scr}$ at baseline	0.003	0.01	-0.02 to 0.03	0.808	0.004	0.01	-0.02 to 0.03	0.798	-0.01	0.01	-0.04 to 0.02	0.507
Urinary NGAL	-	-	-	-	0.81	0.45	-0.06 to 1.70	0.070	-	-	-	-
$eGFR_{Scr}$ at second study visit	-	-	-	-	-	-	-	-	-0.06	0.02	-0.10 to -0.01	0.015
$eGFR_{Scr}$ at third study visit	-	-	-	-	-	-	-	-	-	-	-	-

Abbreviations: SE: standard error, uNGAL: urinary neutrophil gelatinase-associated lipocalin, $eGFR_{Scr}$: estimated glomerular filtration rate based on locally measured creatinine levels.

Continued: Supplementary table 2: Multivariate adjusted logistic regression analysis for eGFR and uNGAL associated with a rapid decline in kidney function at baseline among apparently healthy young males

Factors	Figure 2B				Figure 2C							
	Model 6 with uNGAL levels (n=68)				Model 7 without uNGAL levels (n=73)				Model 8 with uNGAL levels (n=73)			
	Coefficient	SE	95% CI	P-value	Coefficient	SE	95% CI	P-value	Coefficient	SE	95% CI	P-value
$eGFR_{Scr}$ at baseline	-0.01	0.01	-0.04 to 0.02	0.501	0.01	0.01	-0.01 to 0.05	0.328	0.01	0.01	-0.01 to 0.05	0.330
Urinary NGAL	0.54	0.95	-0.57 to 1.65	0.310	-	-	-	-	0.56	0.56	-0.54 to 1.67	0.319
$eGFR_{Scr}$ at second study visit	-0.05	0.02	-0.10 to -0.006	0.025	-	-	-	-	-	-	-	-
$eGFR_{Scr}$ at third study visit	-	-	-	-	-0.06	0.01	-0.09 to -0.02	<0.001	-0.05	0.01	-0.09 to -0.02	0.001

Abbreviations: SE: standard error, uNGAL: urinary neutrophil gelatinase-associated lipocalin, $eGFR_{Scr}$: estimated glomerular filtration rate based on locally measured creatinine levels.

Continued: Supplementary table 2: Multivariate adjusted logistic regression analysis for eGFR and uNGAL associated with a rapid decline in kidney function at baseline among apparently healthy young males

Factors	Figure 2D							
	Model 9 without uNGAL levels (n=67)				Model 10 with uNGAL levels (n=67)			
	Coefficient	SE	95% CI	P-value	Coefficient	SE	95% CI	P-value
$eGFR_{Scr}$ at baseline	-0.004	0.01	-0.04 to 0.03	0.812	-0.004	0.01	-0.04 to 0.03	0.798
$eGFR_{Scr}$ at second study visit	-0.02	0.02	-0.07 to 0.02	0.404	-0.01	0.02	-0.06 to 0.03	0.485
$eGFR_{Scr}$ at third study visit	-0.06	0.02	-0.10 to -0.02	0.002	-0.06	0.02	-0.10 to -0.02	0.002
Urinary NGAL	-	-	-	-	0.44	0.66	-0.86 to 1.74	0.508

Abbreviations: SE: standard error, uNGAL: urinary neutrophil gelatinase-associated lipocalin, $eGFR_{Scr}$: estimated glomerular filtration rate based on locally measured creatinine levels.

Supplementary table 3: Multivariate adjusted logistic regression analysis of factors associated with established renal dysfunction at baseline among apparently healthy young males

Factors	Model 1 (n=74)				Model 2 (n=74)			
	Without uNGAL levels				With uNGAL levels			
	Coefficient	SE	95% CI	P-value	Coefficient	SE	95% CI	P-value
Age	0.14	0.22	-0.29 to 0.57	0.525	0.12	0.22	-0.30 to 0.56	0.563
Season								
Summer	0.0	--	--	--	0	--	--	--
Winter	1.00							
Outdoor work								
Yes	7.14	4.14	-0.97 to 15.26	0.084	7.57	5.42	-3.05 to 18.20	0.162
No	1.00				1.00			
Urinary ACR								
≥30 mg/g	0	--	--	--	0	--	--	--
<30 mg/g	1.00				1.00			
Urinary NGAL	--	--	--	--	3.31	1.87	0.35 to 6.99	0.077
$eGFR_{Scr}$ at baseline	-0.26	0.10	-0.47 to -0.05	0.014	-0.27	0.13	-0.54 to -0.005	0.045

Abbreviations: SE: standard error, uNGAL: urinary neutrophil gelatinase-associated lipocalin, UACR: urinary albumin creatinine ratio, $eGFR_{Scr}$: estimated glomerular filtration rate based on locally measured creatinine levels.

Supplementary table 4: Multivariate adjusted logistic regression analysis of factors associated with a rapid decline in kidney function at baseline among apparently healthy young males

Factors	Figure 2A								Figure 2B			
	Model 3 without uNGAL levels (n=78)				Model 4 with uNGAL levels (n=78)				Model 5 without uNGAL levels (n=67)			
	Coefficient	SE	95% CI	P-value	Coefficient	SE	95% CI	P-value	Coefficient	SE	95% CI	P-value
Age	-0.06	0.06	-0.19 to 0.06	0.354	-0.06	0.06	-0.20 to 0.06	0.327	-0.11	0.09	-0.29 to 0.05	0.187
Season												
Summer	0	--	--	--	0	--	--	--	0	--	--	--
Winter	1.00				1.00				1.00			
Outdoor work												
Yes	1.59	1.08	-0.54 to 3.72	0.144	1.77	1.10	-0.39 to 3.94	0.110	1.42	1.12	-0.78 to 3.63	0.206
No	1.00				1.00				1.00			
Urinary ACR												
≥30 mg/g	0	--	--	--	0	--	--	--	0	--	--	--
<30 mg/g	1.00				1.00				1.00			
Urinary NGAL	-	-	-	-	0.97	0.49	-0.0008 to 1.94	0.050	-	-	-	-
$eGFR_{Scr}$ at baseline	-0.004	0.01	-0.03 to 0.02	0.789	-0.004	0.01	-0.03 to 0.02	0.794	-0.02	0.02	-0.06 to 0.01	0.237
$eGFR_{Scr}$ at second study visit	-	-	-	-	-	-	-	-	-0.06	0.02	-0.11 to -0.01	0.018
$eGFR_{Scr}$ at third study visit	-	-	-	-	-	-	-	-	-	-	-	-

Abbreviations: SE: standard error, UACR: urinary albumin creatinine ratio, uNGAL: urinary neutrophil gelatinase-associated lipocalin, $eGFR_{Scr}$: estimated glomerular filtration rate based on locally measured creatinine levels.

Continued: Supplementary table 4: Multivariate adjusted logistic regression analysis of factors associated with a rapid decline in kidney function at baseline among apparently healthy young males

Factors	Figure 2B				Figure 2C							
	Model 6 with uNGAL levels (n=67)				Model 7 without uNGAL levels (n=72)				Model 8 with uNGAL levels (n=72)			
	Coefficient	SE	95% CI	P-value	Coefficient	SE	95% CI	P-value	Coefficient	SE	95% CI	P-value
Age	-0.11	0.08	-0.29 to 0.06	0.202	-0.18	0.10	-0.38 to 0.01	0.068	-0.18	0.09	-0.37 to 0.01	0.066
Season												
Summer	0	--	--	--	0	--	--	--	0	--	--	--
Winter	1.00				1.00				1.00			
Outdoor work												
Yes	1.48	1.13	-0.73 to 3.71	0.190	3.12	2.01	-0.81 to 7.07	0.120	3.35	2.14	-0.84 to 7.55	0.117
No	1.00				1.00				1.00			
Urinary ACR												
≥30 mg/g	0	--	--	--	0	--	--	--	0	--	--	--
<30 mg/g	1.00				1.00				1.00			
Urinary NGAL	0.63	0.62	-0.58 to 1.84	0.310	-	-	-	-	0.76	0.63	-0.48 to 2.01	0.229
$eGFR_{Scr}$ at baseline	-0.02	0.02	-0.06 to 0.01	0.243	-0.0001	0.01	-0.03 to 0.03	0.996	0.0006	0.01	-0.03 to 0.03	0.972
$eGFR_{Scr}$ at second study visit	-0.05	0.02	-0.10 to -0.004	0.034	-	-	-	-	-	-	-	-
$eGFR_{Scr}$ at third study visit	-	-	-	-	-0.07	0.02	-0.11 to -0.03	<0.001	-0.07	0.02	-0.11 to -0.03	<0.001

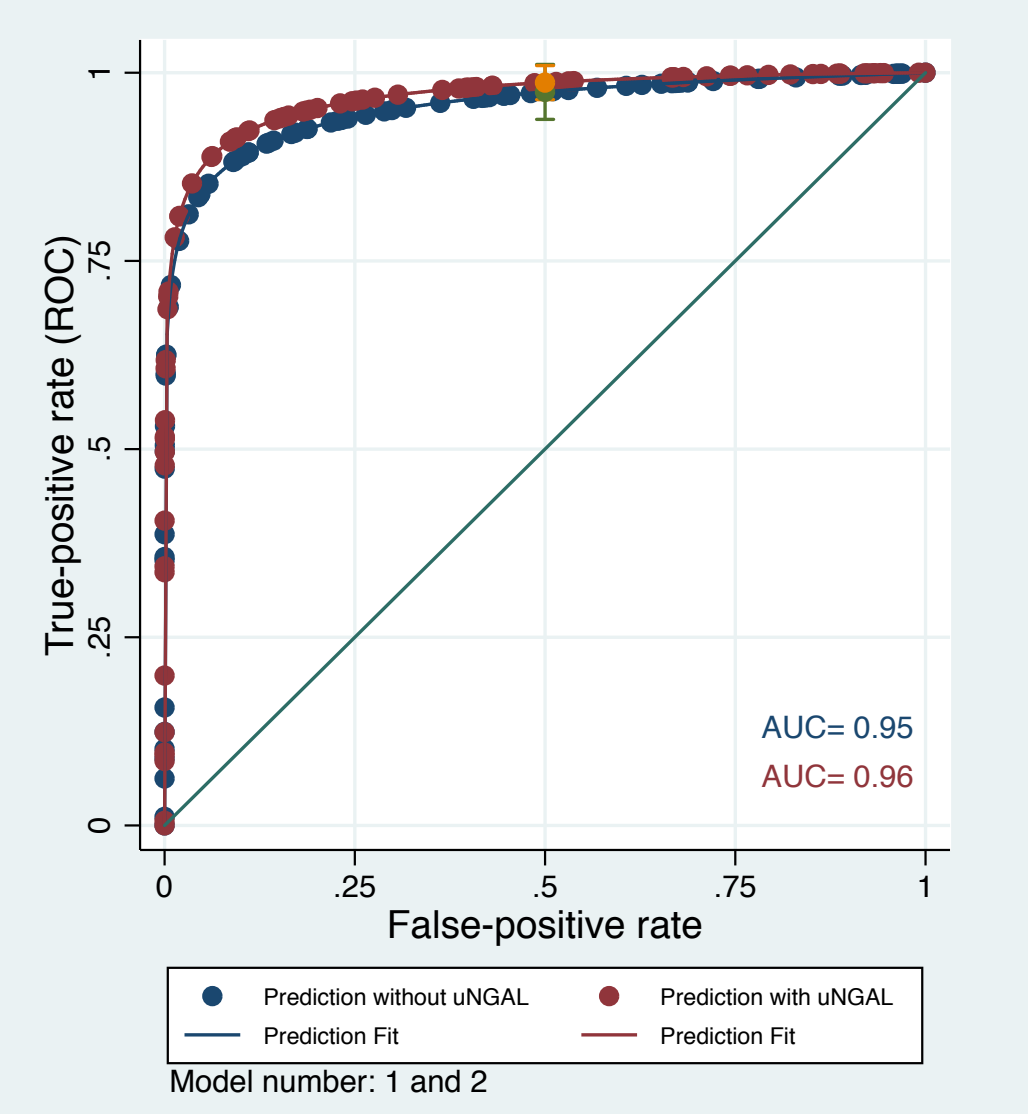
Abbreviations: SE: standard error, uNGAL: urinary neutrophil gelatinase-associated lipocalin, ACR: urinary albumin creatinine ratio, $eGFR_{Scr}$: estimated glomerular filtration rate based on locally measured creatinine levels.

Continued: Supplementary table 4: Multivariate adjusted logistic regression analysis of factors associated with a rapid decline in kidney function at baseline among apparently healthy young males

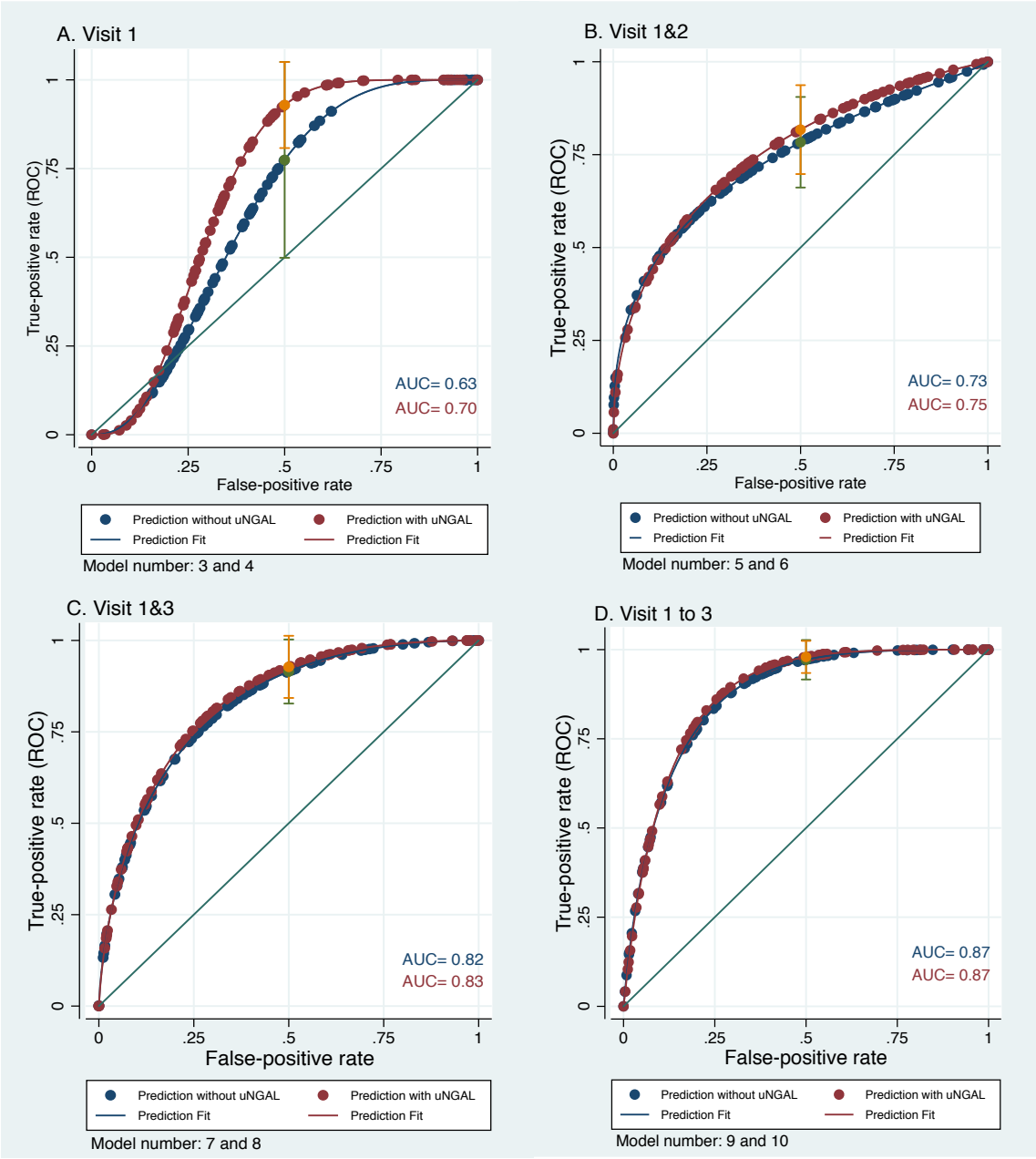
Factors	Figure 2D							
	Model 9 without uNGAL levels (n=66)				Model 10 with uNGAL levels (n=66)			
	Coefficient	SE	95% CI	P-value	Coefficient	SE	95% CI	P-value
Age	-0.25	0.12	-0.50 to 0.002	0.053	-0.23	0.12	-0.49 to 0.01	0.065
Season								
Summer	0	--	--	--	0	--	--	--
Winter	1.00				1.00			
Outdoor work								
Yes	3.40	2.24	-1.00 to 7.81	0.13	3.47	2.29	-1.02 to 7.97	0.130
No	1.00				1.00			
Urinary ACR								
≥30 mg/g	0	--	--	--	0	--	--	--
<30 mg/g	1.00				1.00			
Urinary NGAL	-	-	-	-	0.42	0.73	-1.01 to 1.86	0.561
$eGFR_{Scr}$ at baseline	-0.02	0.02	-0.07 to 0.01	0.239	-0.02	0.02	-0.06 to 0.01	0.254
$eGFR_{Scr}$ at second study visit	-0.03	0.02	-0.08 to 0.02	0.269	-0.02	0.02	-0.08 to 0.03	0.360
$eGFR_{Scr}$ at third study visit	-0.08	0.02	-0.12 to -0.03	0.001	-0.07	0.02	-0.12 to -0.03	0.001

Abbreviations: SE: standard error, uNGAL: urinary neutrophil gelatinase-associated lipocalin, UACR: urinary albumin creatinine ratio, $eGFR_{Scr}$: estimated glomerular filtration rate based on locally measured creatinine levels.

Supplementary figure 2: ROC curves for the model predicting stable kidney function versus established renal dysfunction. The 95% confidence intervals for the ROC curves (0.5) are displayed.



Supplementary figure 3: ROC curves for the model predicting stable kidney function versus a rapid decline in kidney function. The 95% confidence intervals for ROC curves (0.5) are displayed.



Chapter 6: Overall discussion

The core of the thesis has been the first community-based cohort study among apparently healthy young people in Nicaragua. In addition, I learned to use a number of other epidemiological approaches to advance our understanding of CKDu, including a systematic review and a community-based prospective cohort study, as well as a nested case-control study.

The specific objectives of the thesis (1.4.3) were: (1) to review the current knowledge and gaps in our understanding of the potential causes of CKDu in the Pacific coast of Central America; (2) to understand which risk factors are associated with decline of eGFR among a healthy young population at risk of developing CKDu; and (3) to determine if repeated routine creatinine tests combined with baseline information (age, season, and work performed) and measurements of urinary neutrophil gelatinase-associated lipocalin (uNGAL) levels and the urinary albumin-creatinine ratio (UACR) can identify the subgroup of individuals at risk of a future rapid decline in kidney function. Addressing objective 3 will enable researchers to identify a clinical case definition that can be used in further epidemiological studies.

The main findings will be summarized and discussed in terms of the overall strengths and limitations of the approaches used **(Sections 6.1 and 6.2)**. The clinical and public health implications of the research (individual/family, medical, government/healthcare system and industry implications) will be described in **section 6.3**. The academic research implication and future research questions

will be presented in **sections 6.4 and 6.5**. Finally, an overview of my personal learning and conclusions are discussed in **sections 6.6 and 6.7**.

6.1 Summary and synthesis of the research findings

6.1.1 What was already known about this topic

In the last two decades, chronic kidney disease of unknown origin (CKDu) has emerged as major public health problem in Mesoamerica. Excess mortality due to CKDu among young agricultural workers has been reported in many countries across the region.[12, 13, 16, 33, 104-106] This disease has some unique characteristics, including its lack of association with well-established causes of kidney disease in developed countries, such as diabetes, hypertension and glomerulonephritis. Furthermore, according to epidemiological studies, CKDu is mostly prevalent (but not restricted to) in young male agricultural workers who are often in their 30s and are geographically distributed along the Pacific coast in Mesoamerica. Positive correlations have been identified with certain factors, such as the male sex, low altitude, agricultural work, and high water intake.[12, 14, 101, 106, 108, 109] The clinical manifestations include minimal proteinuria, normotensive, anaemia, electrolyte disorders (hyponatremia and hypokalaemia), hyperuricaemia, and small echogenic kidneys.[22, 23, 27] The clinicopathological picture involves tubulointerstitial damage and glomerular enlargement.[28, 29, 49, 119]

The specific causes and aetiological pathways remain to be identified. The hypothesized causes of CKDu include cyclical dehydration episodes linked to extreme working conditions (heat stress and physical exertion) plus an inadequate hydration pattern/solution that possibly occurs concomitantly with

other factors, such as self-medication (nonsteroidal anti-inflammatory drugs), infectious diseases (leptospirosis, hanta and dengue), environmental toxins (pesticides, heavy metals, and mycotoxin) and genetic susceptibility, since entire families are affected in some communities.[12, 13, 76, 101, 120, 121] However, substantial knowledge gaps and an absence of empirical evidence regarding the natural history of the disease over time and the cause(s) of CKDu remain.

6.1.2 What the study adds

Upon reviewing the literature, I concluded that the current knowledge is still limited, because studies have not identified the natural history and cause(s) of the disease. My systematic review showed that cross-sectional studies and occupational longitudinal studies have been affected by methodological problems, which include misclassification, reverse causation, a substantial of loss follow-up that affected the study power, and short follow-up periods (6 months). These limitations may account for the inconclusive findings reported to date.[122]

I was involved in establishing the first community-based cohort study dedicated to studying this issue. We attempted to recruit healthy young adults without CKD at baseline. We excluded 5% of men in the target age group who reported a diagnosis of kidney disease at baseline; this prevalence is approximately one hundred times higher than the CKD prevalence for this age group in the UK.[123] In total, 350 participants were followed for two years. The descriptive analysis of eGFR slopes over time is shown below (Figure 1). A normal distribution of slopes with a mean of approximately 0 is observed, but at the lower tail, a second cluster, or “bump”, in a subgroup with rapidly declining kidney function

at -20 mL/min/1.73 m^2/year is observed. This second cluster almost certainly reflects individuals experiencing the early stages of MeN.

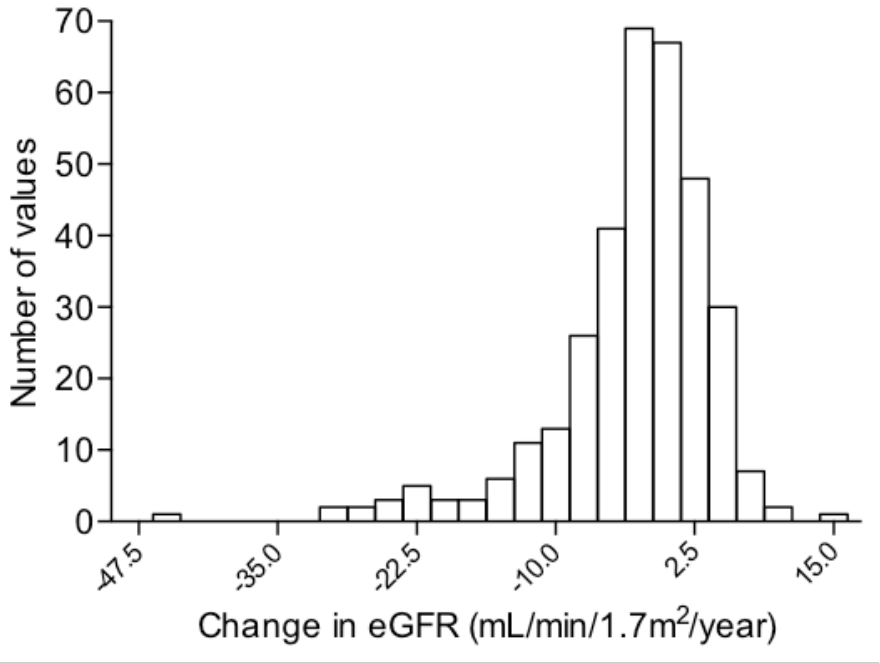


Figure 1: Distribution of eGFR trajectories over the two-year follow-up in the study population

This distribution of decreasing eGFR slopes over time explains why the multivariate analysis did not display a good fit: the slopes of eGFR over time were not normally distributed. In response to this limitation, the growth mixture model helped us to address the non-normal distribution of eGFR slopes. Three distinct groups of trajectories of decline in kidney function in males (normal kidney function, rapid decline in kidney function and establish renal dysfunction) and two in females (normal kidney function and rapid decline in kidney function) were identified. The male group with a rapid decline in kidney function was currently involved in agricultural work and outdoor work with a lack of shade availability during work breaks, but not any other particular risk factor. On the other hand, the few women that experienced a rapid decline in kidney function

did not report historical or current outdoor or agricultural work or indeed any other risk factor.[124] Based on this finding, different aetiologies may affect different groups, e.g., infectious disease, phyto/mycotoxins or a poor water supply, etc., or alternatively, a causal exposure spread across the population but caused more cases of disease (through increased risk of exposure or exacerbating factors) in men.

Importantly, we do not know whether only 10% of the population are affected, or whether other people with previously stable kidney function would enter this rapid decline group with a longer observation period.

The meta-analysis identified significant positive correlations between the male sex, lowland altitude, water intake and a family history of CKD with CKDu.[122] Most of the epidemiological studies have consistently reported strong correlations between the male sex, lowland altitude and CKDu.[15, 18-20, 24, 30-32, 45, 69, 106, 112, 116] Additionally, in our community-based cohort study, 10% of men experienced an unprecedented decline in kidney function over the 2-year follow-up period compared to women (3.5%).[124]

The association with high water intake is difficult to interpret because some studies have reported a positive correlation between low water-intake or high water-intake.[30, 69, 110] This association may be interpreted as refuting or supporting the hypothesis that dehydration is a risk factor for CKDu due to volume depletion during heavy physical work or it may simply reflect a reverse causation. In this context, reverse causation means that individuals with tubular

damage may not be able to concentrate urine, and therefore the high water-intake may simply be a sign of early kidney damage.[122, 125]

Although many cross-sectional studies and two systematic reviews have described a positive correlation between a family history of CKD and CKDu,[20, 32, 69, 120, 122] this correlation was not reported in a recent community-based cohort study of young adults, where the family history of CKD does not appear to be a risk factor for a rapid decrease in eGFR, because the analysis was adjusted for age, education level and community.[124] It is often assumed that the clustering of a disease in families is due to genetic diseases, such as autosomal dominant polycystic kidney disease (ADPKD) and APOL1 gene variations.[126-129] However, diseases frequently cluster in families because families share disease risk factors. Thus, these genetic diseases may not explain the excess incidence of CKDu in a family cluster.

Our community-based study identified a number of key risk factors that were positively correlated with a rapid decline in eGFR: outdoor work, agricultural work and a lack of shade availability during work breaks, reported at baseline.[124] These findings are consistent with the results reported by other epidemiological studies, which described that decreased kidney function was associated with agricultural work (sugarcane, banana, coffee, and subsistence farming).[15, 19-21, 24, 25, 31, 32]

On the other hand, neither the systematic literature review nor our community-based study found evidence of associations of a loss of renal function with heat

stress, agrochemical exposure, alcohol consumption, and non-steroidal anti-inflammatory drug uses.[122, 124] All of the above risk factors have been proposed as the main causes for CKDu in many of the publications.[21-24, 26, 35, 39-41, 45, 46, 51, 59-61, 69, 118, 130-133] However, the findings from the community-based cohort study, studies conducted at mill industries indicate that sugar cane work induces small increases in serum creatinine levels across the work day or across the harvest season, with authors suggesting that this change reflects an AKI secondary to heat stress.[22, 23, 43, 50] Along with kidney damage, these changes in the creatinine level may reflect a dehydration process that reduces the total water content (leading to all solutes being concentrated), increased creatinine production (as a consequence of high protein intake or muscle catabolism) or a physiological haemodynamic reduction in GFR. The observation that other kidney biomarkers did not show an increase in across shift testing and instead increased across seasons[12, 23, 35, 43, 125, 130, 134] suggests that cellular injury may not underlie the across shift changes in creatinine levels that occur throughout the work day. To date, robust evidence for a causal link between a permanent reduction in kidney function and heat stress is unavailable.[52]

Furthermore, heat stress exposure has also been reported to reduce work capacity, decrease productivity and increase occupational accidents.[130, 135] Reduced productivity was reported among construction workers, miners, steel workers, etc. worldwide.[136-140]

Our data suggests that self-report of exposure to agrochemicals was not a major risk factor for a decline in kidney function, and we have not found

evidence for a role for heavy metals in this disease.[141] These findings are consistent with the current body of evidence, which has not identified an association with reduced kidney function in Mesoamerica.[20, 21, 31, 62, 69, 110, 122] However, a study conducted among licensed pesticide applicators in the US has described a positive correlation with highest tertile of accumulated lifetime days of exposure to certain pesticides (atrazine, pendimethalin, metolachlor, alachlor, and paraquat), and ESRD after adjusting for age and state, but not social inequality or diabetes and smoking history and other risk factors, which may associated with the duration of work as a pesticide applicator.[63] Another study in a urban population within previous exposure to pesticides in Delhi, India, found that blood levels of organochlorine pesticide metabolites, such as gamma-hexachlorocyclohexane, p,p'-dichlorodiphenyltrichloroethane, and beta-endosulfan, were associated with CKDu compared to healthy controls after adjusting for age, sex, BMI, and the total lipid profile. Again, the study did not adjust for inequalities that may drive exposure to pesticides and may explain the apparent association with CKDu.[142] Ultimately, better designed studies are needed, as the existing pesticide studies in the US, India and Sri Lanka are likely confounded by incomplete adjustment for socio-economic status, chronic disease, unhealthy behaviours and other risk factors.

To aid in developing a clinical case definition, I assessed urinary neutrophil gelatinase-associated lipocalin (uNGAL) levels as an additional marker for the early identification of the rapid decline group. The prediction score from the model including only one eGFR measurement plus uNGAL levels showed an improved ability to detect individuals at risk of a rapid decline in kidney function

(AUC: 0.75) compared to the model without uNGAL levels (AUC: 0.51). However, when three eGFR measurements were added, the discrimination power was almost the same for the model with and without uNGAL levels (AUC: 0.89 and AUC: 0.88 respectively). Thus, uNGAL levels do not help distinguish people at risk of decline in kidney function when serial measurements of eGFR (baseline, 6 and 12 months) are available. Hence, the latter strategy is preferable, rather than expending resources measuring uNGAL levels. In addition, for the identification of individuals with established renal dysfunction, a single measurement of eGFR or uNGAL levels was sufficient to identify membership of this subgroup, resulting in a well-fitting prediction model (AUC: 0.98).

In summary, this study has contributed to the knowledge base of CKDu by describing the natural history of a highly progressive form of kidney disease among men in northwest Nicaragua. It also highlights the urgency in establishing larger community-based cohort studies in different settings and intervention studies to examine the cause(s) and reduce the progression of CKDu. Cross-sectional studies are useful for understanding a static disease, but based on the data obtained from this cohort, the disease has a strong temporal element. Furthermore, due to their very nature, cross-sectional studies may not capture the group with a rapid decline in kidney function. Thus, this group will be misclassified in a cross-sectional study.

Notably, occupational studies are affected by the healthy worker effect because mill industries only hire workers with "normal" kidney function (Scr <1.3 mg/dl)

for the harvest period. Hence, when workers have an abnormal Scr test, they lose their jobs and will move to other occupations, such as banana plantation work or subsistence agriculture. Hence, in the context of screening of workers for CKD, cross-sectional studies are unable to quantify the real magnitude of this disease and address reverse causation.

6.2 Strengths and Weaknesses

Individual strengths and limitations of each individual paper are described in **Chapters 2 to 5**. The overall strengths of this thesis are presented below.

6.2.1 Strengths

6.2.1.1 Study design

This thesis is based on the first-ever community-based prospective cohort study among an apparently healthy young population that has assessed the natural history of and factors associated with decline in kidney function over a two-year follow-up period. A census of nine communities was performed in the population aged 18-30 years, and individuals without any pre-existing diagnosis of diabetes, glomerulonephritis or hypertension were invited to participate in the study. All apparently healthy men were recruited and a random sample of all eligible women was selected.

6.2.1.2 Community engagement and study retention

Community engagement with community leaders and participants was conducted before and during each study visit to achieve a participant retention

rate of 92% over the two-year follow-up period. Participants received their lab results within a week of the study visit to enhance community engagement.

6.2.1.3 Single batch measurement and use of the eGFR trajectory as the outcome measure

Analyses of serum creatinine and cystatin C levels were performed in a single batch at end of the study in Oxford, UK. Samples were analysed using quality control methods referenced to international standards. For example, the reference method for creatinine was isotope dilution mass-spectrometry (IDMS). The outcome of the follow-up was the eGFR trajectory calculated for each participant at each study visit from the growth mixture model for men and women. This strategy reduced misclassification and robustly identified individuals affected by MeN.

6.2.2 Limitations

This section presents the overall limitations of this thesis, along with the potential impact in terms of the lack of associations between certain exposures and decline in kidney function.

6.2.2.1 Selection of the sample

A community-based census was conducted among young adults aged 18-30 years. The census identified 520 adults, which represent approximately 10% of the total population, but if we compare these data with Nicaragua population pyramid, fewer young adults live in these villages than might be expected.[143] Several explanations for this discrepancy are possible. First,

as is typical for communities in many LMICs, young adults migrate to cities or other regions in pursuit of better job opportunities and quality of life.[144] Second, the overall population is small and many young adults have died due to CKDu. Finally, the national census data may be inaccurate, as they were last updated in 2005.[143]

However, the entire healthy adult population in the target age range was recruited from the affected villages, and therefore associations with a decline in kidney function will be internally consistent, even if they are not necessarily generalizable to other regions.

6.2.2.2 Exposure assessment

6.2.2.2.1 Occupational exposure

Work history was assessed by self-report at baseline. These data were collected by asking participants about their entire working life (e.g., What was your first occupation and for how many years have you been working in that occupation? How many hours per day did you work?). Although we used these detailed data collected at baseline, we were unable to establish a relationship between a previous occupation and a decrease in eGFR. This lack of an association may be because none exists, or because participants reported short-term exposure to multiple different occupations during their work life.

In addition, a discrepancy was observed with the current occupation in the sensitivity analysis at the second visit compared with the baseline visit, but this can be explained by seasonal differences in exposures. As

seasons change, so do the risk factors. Trouble is that participants who lost kidney function between visit 1 and 2 may have changed behaviour as result of the kidney function test results, introducing reverse causality. Hence, results have to be interpreted with caution.

6.2.2.2.2 Heat exposure

6.2.2.2.2.1 Occupational heat exposure

The evidence base addressing the health effects of occupational heat stress exposure has increased rapidly in recent years.[37-39, 47, 139, 145] A range of occupations (outdoor and indoor) are known to be associated with high levels of heat stress. Outdoor workers at risk of heat stress exposure include sugarcane workers (cane/seed cutters, seeders, pesticide irrigators, and water irrigators), miners, agricultural workers, construction workers, brick-makers, and military personnel.[22, 24, 26, 38-41, 43, 45, 136, 146] Meanwhile, indoor workers exposed to heat stress include bakery workers, steel workers, factory workers, etc.[139, 140, 147]

An objective assessment of heat stress is not a straight forward measurement. In Central America, heat exposure has been assessed by measuring the wet bulb globe temperature (WBGT) or using the heat index at the workplace.[40, 41, 147-150] Both measurements estimate the heat effects by quantifying the air temperature, humidity, wind speed and radiant heat.[40, 41, 147-150] For example, Crowe et al measured the heat stress exposure among manual cane cutters in Costa Rica by measuring the WBGT index and reported that cane cutters are working under excessive heat (WBGT>26ºC) for most of the work day.[40, 41] In El

Salvador, approximately 40% of coast cutters worked and approximately 10% cutters at a height of 400 mts worked at temperatures above 30°C (WBGT) during the 2014-2015 harvest.[130]

In addition, a recent pilot study in a subgroup of cane cutters in El Salvador reported that their average heart rate (HR) was 54% of the maximum HR during one day of work, a higher frequency than soldiers participating in multi-day exercises. Their average core body temperature was 37.5ºC (95% CI: 37.7 to 37.4ºC) during a work day averaging a temperature of 38.4ºC (95% CI: 38.7 to 38.1ºC). For two of 14 workers, this value exceeded 39.0ºC.[151] These severe changes in HR and body temperature suggest that cane workers are exposed to extreme heat stress due to high intensity muscular work in a hot and humid climatic environment. These changes activate heat loss mechanisms by sweating; however, sweating may impact the hydration status and potentially renal function.

6.2.2.2.2.2 Self-reported heat stress

A number of scientific tools to assess self-reported heat stress among outdoor workers have been developed. These tools include semi-quantitative environmental parameters that have been validated using physiological measures.[152-154] For example, a study evaluating the heat stress perception index (perceived exertion and thermal sensation) among construction workers in India reported a good correlation between the self-reported index and environmental and physiological variables:

heart rate and WBGT.[155] Our findings, which were obtained using similar questions as in this index, suggested that self-reported heat exposure was not a risk factor for decline in kidney function. This discrepancy might be because the questions are not valid in the study population or because self-reported heat stress is not associated with a decline in kidney function.

6.2.2.2.2.3 Symptoms related to heat

According to recent reports, headache, weakness, dizziness, fever, nausea, tachycardia, fatigue, muscle cramps, and vomiting are associated with heat stress among sugarcane workers in Central America.[42] An assessment of these symptoms is based on protocols from the US Army, which has used these variables to assess the health impact of heat exhaustion among soldiers with high physical demands.[156] However, these symptoms are not specific to heat exhaustion because they are also induced by other diseases. Based on our data, heat-related symptoms captured by self-report are not associated with a decline in kidney function. Thus, a heat-related symptom questionnaire must be validated and/or the novel biomarkers that are able to capture physiological heat stress on an individual level must be identified.

6.2.2.2.3 Agrochemicals

Long- and short-term pesticide exposures were assessed with a questionnaire. However, farmers quite often did not remember the type of

pesticide, when or how much was applied. Similar to heat stress, validated questionnaires to quantify pesticide exposure at work are lacking. Despite this limitation, this thesis included the self-reported pesticide exposure as part of an exploratory analysis of CKDu, but no clear association was detected in this analysis. Future studies measuring pesticide metabolites in urine are needed to overcome problems of identification, dose and possible recall bias.

6.2.2.3 Measurement error in eGFR

A major challenge in epidemiological kidney research is errors in GFR estimates due to both biological variability (within-person and between-person) and measurement errors in serum creatinine levels. Intraindividual variations in eGFR have been observed and well-documented among healthy and unhealthy people because kidney function varies during the day. This variation is caused by multiple factors, such as strenuous exercise, high animal protein meal intake, and changes in plasma volume. Inter-individual variations in eGFR also arise from non-renal factors, e.g., sex, genetic background and muscle mass. All of these factors affect creatinine production and secretion.[6, 8] For example, a cane cutter performs strenuous physical effort and work under hot environmental conditions. He may have intermittent meat consumption (leading to variability in creatinine levels) and a higher muscle mass than others (or from the beginning to end of the harvest season), leading to a bias in eGFR estimates, and experience immediate changes in kidney function in response to loss of fluid, which may lead to transient increases in serum creatinine concentrations after work. Therefore, the small increases in serum creatinine

observed in sugarcane workers[22, 23, 26, 43, 45] may not reflect sources of variation other than short-term changes in kidney function and should be treated with caution.

Cystatin C is less susceptible to changes in diet/muscle mass compared to creatinine and thus might be a more useful marker in short-term studies of eGFR in workers.[157, 158] The eGFR calculated based on both serum creatinine and cystatin c levels was measured repeatedly in the same person over time and assayed in a single batch at the end of the study to avoid some of these problems in our cohort.

The eGFR was estimated using a validated IDMS-traceable method and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. However, the CKD-EPI formula has not been validated for Latin American or Mesoamerican populations to date.[2] This lack of validation may lead to misclassification error due to over or underestimation of the eGFR. However, because repeated measurements of serum creatinine and cystatin c levels were performed over time to estimate rate of decline in kidney function, the main comparison in our cohort was based on within-person changes.

6.3 Clinical and public health implications

CKDu is a silent disease in Mesoamerica that affects thousands of individuals among the economically active population, causes the premature death of young adults, and has socioeconomic implications for patients, families, communities, local industries and health services.

6.3.1 Individual/family implications

The living conditions of the inhabitants of northwest Nicaragua are largely determined by the low education level and limited employment opportunities. Work poses significant health risks due to the psychosocial pressure and economic uncertainty generated by precarious employment. The working environment appears to be a major health hazard. The nature of the employment contracts (piecemeal work and temporary contracts) and the availability of labour (high rates of unemployment with a limited labour market providing few alternatives) make the workers opt for jobs where working conditions (high temperatures and heavy physical loads), in many cases, would be classed as inhumane in most countries around the world in contemporary times.

The consequences of a CKDu diagnosis are also severe. For example, when a worker is diagnosed with CKDu, it has a tremendous impact on his and his family's lives because he will experience decreased physical and mental function, leading to a worsening of the socioeconomic status, disability, family dysfunction, early retirement and premature death. Indirectly, this situation will promote school drop-out at an early age and increase child labour to help provide for the economic needs of the family, leading to a vicious cycle of health-related poverty and disease as result of poverty. A question for further research is whether children whose parent died early as a result of CKDu may be predisposed to developing CKDu as

result of child labour in precarious working conditions superimposed on physiological changes in kidney function that occur during puberty.[159]

6.3.2 Medical implications

CKDu has many implications for health providers and services. First, physicians from hotspot regions are diagnosing patients with CKDu late in the course of disease, e.g., at a time when they need renal replacement therapy (RRT).[160, 161] This late diagnosis is partially due to the lack of a CKD screening surveillance system at the primary, secondary or tertiary health care level. In addition, a lack of continuing professional education in the area of CKD has been noted, and practitioners are thus unaware of the current CKD definition, classification and requirements for RRT when patients have end-stage renal disease.[162]

Second, a serious concern about the best treatment option to prevent the progression to end-stage renal disease (ERSD) has been reported. For example, based on a personal communication with Dr. Ramón García-Trabanino[1] from El Salvador, he has been conducting an empirical trial for more than 10 years that aims to slow the progression of CKDu. This protocol consists of bicarbonate and a low dose of allopurinol, and he has observed a deceleration of the progression to ERSD and an apparent alleviation of dysuria, which is very common in the early stages of CKDu. Any benefits for

[1] Dr. Ramón García-Trabanino; Centro de Hemodiálisis, San Salvador, El Salvador; and Fondo Social de Emergencia para la Salud, Centro Monseñor Oscar Arnulfo Romero, Cantón Tierra Blanca, El Salvador.

patients receiving this type of treatment remain unknown, as there is no empirical evidence for this type of therapy.

6.3.3 Government/healthcare system implications

The healthcare system in Nicaragua is divided into the public health system (MINSA) and private health insurance. MINSA has the responsibility of providing access and medical care to the general population and informal workers (representing 80% of the working population that are not covered by the Nicaraguan Institute of Social Security (INSS)). INSS provides health care only to formally employed workers from large companies and the public sector, which represent between 20 to 22% of the working population.[163]

MINSA has implemented substantial efforts to address the epidemic of CKDu in the region by opening eight small-medium-sized haemodialysis units (293 patients are receiving three sessions of haemodialysis per week) at MINSA and six small units for continuous ambulatory peritoneal dialysis in the hotspot area (227 patients are receiving this treatment option), but this effort is still insufficient given the rates of CKDu in northwest Nicaragua.[161, 162] On the other hand, the INSS has admitted 3050 patients with CKD to the RRT programme from 2008-2017.[163]

Despite these improvements, resources for the diagnosis and treatment CKD or CKDu are insufficient. For example, Nicaragua has the lowest number of nephrologists per million population (4.6 nephrologists per million population) in Central America, as well as a lack of nurses,

nephropathologists and other personnel trained in nephrology.[161] Remarkably, although the country has the highest mortality rate for CKDu in Mesoamerica, a substantial unmet need in the nephrology workforce persists.

In addition, the infrastructure (nephrology centres with haemodialysis and peritoneal dialysis units) that manages patients with CKDu is insufficient and inadequate. In addition, problems with the supply of medications and laboratory capacity have been noted, indicating that patients with pre-end stage renal disease may not be diagnosed and complications are not treated. Indeed, the system is already so stretched and RRT is so expensive that the budget for additional preventative care measures is limited.[161, 162] Finally, a CKD surveillance system, health promotion and prevention strategies for CKDu at the community level are lacking.

6.3.4 Industry implications

The sugar mills started to implement a screening test prior to hiring workers in 2001. This programme allows the sugar mills to identify potential workers with normal and non-normal serum creatinine (Scr) levels. The Scr reference value that the industry established was initially ≤1.2 mg/dL of creatinine (this represents an eGFR of 87 mL/min/1.73 m^2 for a 20-year-old man).[45] Due to the low number of people with a Scr level below the cut-off point, a new

reference value was defined last year (≤1.3 mg/dL)[2] (representing an eGFR of 79 mL/min/1.73 m^2 for a 20-year-old man).

Based on the high number of sugarcane workers with a high creatinine level at the end of the harvest, the industries have decided to mechanize the harvest. Currently, mechanical harvesting has increased to 90% of the sugarcane crop in one of biggest mills in Nicaragua and approximately 70% in other sugar mills. Thus, sugarcane industries are hiring fewer manual workers (cane cutters) during the harvest.[164] However, the impact of increasing mechanisation on the availability of jobs is negative because the unemployment and poverty rates will increase in the rural areas due to the lack of other employment opportunities.

In addition, sugarcane industries have taken action to reduce the heat stress and dehydration in sugarcane workers by implementing an intervention programme (Worker Health and Efficiency (WE) programme) during the harvest season in El Salvador and Nicaragua.[130] This intervention involves providing water, rest and shade together with an ergonomic machete and breathable clothing. In addition, health education and field monitoring occurs during the work day. This intervention programme has not been evaluated systematically nor implemented on a large-scale.[135, 164] Nevertheless, at least some parts of the sugar cane industry are aiming to improve working conditions and to mitigate possible effects of heat stress and heavy workload

[2] *Personal communication Denis Chavarria, Department of Occupational health and Safety, Ingenio San Antonio*

on sugarcane cutters. However, to date, evidence for any possible protective effects on kidney function are limited.[135, 164]

6.4 Academic research implications

The cause of CKDu in Nicaragua may be so highly prevalent that it is difficult to detect using a relatively small and localized cohort study (i.e., all or almost all community members are exposed to the primary cause of disease). This hypothesis supports the idea of establishing a number of cohorts in different settings with a wider range of exposures. Therefore, a useful strategy would be to design and implement a generic cohort protocol for detecting progressive kidney disease in rural LMIC communities (for a draft see appendix C of this thesis). These studies might also provide the platform for targeted clinical trials of individuals with a progressive disease and contribute to better insights into the progression, causation, and methods to delay the transition to ESRD.

6.5 Future research

The work presented in this thesis does not provide evidence for subjective measurements of exposure to identify hypothesized risk factors. Based on these findings additional analyses have been done that are not part of this thesis. For example, we have measured heavy metals in drinking water and urine samples, pesticide metabolites and Ochratoxin A (OTA); a manuscript is currently in development based on these measurements.

We were unable to identify the exposures associated with a decline in eGFR in this population during the first two years of follow-up. These could be due to a

relatively short follow-up period, the moderate sample size that was recruited in the first phase of this cohort or that the questionnaire did not capture the extent of exposure. That is why we decided to expand and extend our existing community-based longitudinal study from 2018 to 2020 and allow us to continue describing the natural history of and exploring the causality of this disease by using a more refined exposure matrix. In this second phase, we are following the existing 350 participants and recruiting to further 250. The participants will be visited once a year.

A biobank has been developed at the Centre for Nephrology at University College London (UCL) for future analysis such as genetic, metabolomics and proteomics analysis, infectious diseases, etc.

Future research efforts should address a number of questions:

- The development and validation of a core questionnaire to capture occupational and non-occupational exposures, such as sociodemographic data, labour history, heat stress and hydration symptoms, medications, agrichemical exposure, etc., in different contexts and countries.
- Validation of exposure biomarkers (e.g., for heat stress) or other tools (e.g., wearables) in different locations, both at the workplace and community levels.
- Validation of equations used to calculate eGFR in local populations in various regions of the world.

- Studies of infectious disease incidence and associations with eGFR would help researchers explore the potential role of leptospirosis, dengue or chikungunya on the genesis of CKDu.
- Studies that investigate family members and offspring of families affected and not affected by CKDu, including the role of child labour and careful phenotyping during adolescence to capture the timing/role of puberty.
- Clinical trial protocols with promising treatments known to delay the loss of kidney function in other settings, e.g., oral bicarbonate and allopurinol therapy, or only allopurinol. This method requires the identification fast progressors to target interventions.
- Larger community-based and occupational cohort studies at different locations and with different populations are needed, as described in the previous section.
- More studies of social health determinants in varying contexts and countries are needed to identify how these factors interact with occupational behaviours.

6.6 Personal learning

My doctoral training has been a challenging journey in my life for many reasons. During the registration process, I realized that my English skills were insufficient to enrol in a PhD programme. Thus, I took an intensive English course to improve my writing and speaking to surmount this obstacle.

I have gained new knowledge and skills in the use of the STATA software for first time, and during the course of my studies, I have learned to conduct

complex analyses of longitudinal data (multivariate analyses, growth mixture modelling analyses) using STATA software, which were totally new concepts to me. Thus, I have acquired new theoretical and practical knowledge of the challenges of handling longitudinal data, analysing time-dependent data and interpreting longitudinal data.

I have developed the skills to conduct systematic reviews and meta-analyses for first time in my life by conducting a literature review on my research topic. These skills led me to sharpen my critical thinking by very carefully scrutinising scientific papers to identify the strengths and limitations (bias) of each study.

I have gained knowledge on how to deal with and address reviewers' comments during the peer review of my manuscripts.

I have learned that although many research studies have been conducted in Mesoamerica, the current evidence on the cause(s) is similar compared to the state of knowledge ten years ago. Most of the occupational studies have been affected by insufficient or short-term follow-up data and by the healthy worker effect. This problem cannot be solved by conducting cross-sectional studies in the region.

6.7 Conclusions

To my knowledge, this set of studies is the first body of work to describe the natural history of and factors associated with declining kidney function in a population at risk of developing CKDu in Nicaragua. The cohort study has shown the importance of repeated kidney function assessments to characterize the loss of kidney function in this high-risk population. This research was unfortunately unable to identify the cause(s) of this silent and mysterious disease. Thus, better exposure data are needed to obtain a better understanding of the aetiology. Therefore, this cohort study should be replicated in other communities using improved exposure assessment tools and across a wider range of populations as soon as possible.

References

1. KDIGO: **KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease**. *Kidney Int Supps* 2013, **3**(1):1-150.
2. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, Kusek JW, Manzi J, Van Lente F, Zhang YL *et al*: **Estimating glomerular filtration rate from serum creatinine and cystatin C**. *N Engl J Med* 2012, **367**(1):20-29.
3. Cockcroft DW, Gault MH: **Prediction of creatinine clearance from serum creatinine**. *Nephron* 1976, **16**(1):31-41.
4. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D: **A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group**. *Ann Intern Med* 1999, **130**(6):461-470.
5. Levey AS, Coresh J, Greene T, Marsh J, Stevens LA, Kusek JW, Van Lente F, Chronic Kidney Disease Epidemiology C: **Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values**. *Clin Chem* 2007, **53**(4):766-772.
6. Levey AS, Greene T, Schluchter MD, Cleary PA, Teschan PE, Lorenz RA, Molitch ME, Mitch WE, Siebert C, Hall PM *et al*: **Glomerular filtration rate measurements in clinical trials. Modification of Diet in Renal Disease Study Group and the Diabetes Control and Complications Trial Research Group**. *J Am Soc Nephrol* 1993, **4**(5):1159-1171.
7. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T *et al*: **A new equation to estimate glomerular filtration rate**. *Ann Intern Med* 2009, **150**(9):604-612.
8. Padala S, Tighiouart H, Inker LA, Contreras G, Beck GJ, Lewis J, Steffes M, Rodby RA, Schmid CH, Levey AS: **Accuracy of a GFR estimating equation over time in people with a wide range of kidney function**. *Am J Kidney Dis* 2012, **60**(2):217-224.
9. Matsushita K, Ballew SH, Astor BC, Jong PE, Gansevoort RT, Hemmelgarn BR, Levey AS, Levin A, Wen CP, Woodward M *et al*: **Cohort profile: the chronic kidney disease prognosis consortium**. *Int J Epidemiol* 2013, **42**(6):1660-1668.
10. van der Velde M, Matsushita K, Coresh J, Astor BC, Woodward M, Levey A, de Jong P, Gansevoort RT, Chronic Kidney Disease Prognosis C, van der Velde M *et al*: **Lower estimated glomerular filtration rate and higher albuminuria are associated with all-cause and cardiovascular mortality. A collaborative meta-analysis of high-risk population cohorts**. *Kidney Int* 2011, **79**(12):1341-1352.
11. KDIGO: **KDIGO Clinical Practice Guideline for Acute Kidney Injury**. *Kidney Inter Supps* 2012, **2012**(2):1-138.
12. Wegman D, Crowe J, Hogstedt C, Jakobsson K, Wesseling C, editors: **Mesoamerican nephropathy: report from the second international research workshop on MeN**. In: *SALTRA.* Edited by Central American Institute for Studies on Toxic Substances (IRET-UNA) and Program on Work Environment and Health in Central America (SALTRA), vol. 33. Heredia, C.R: SALTRA/IRET-UNA; 2016.
13. Wesseling C, Crowe J, Hogstedt C, Jakobsson K, Lucas R, Wegman D, editors: **First international research workshop on mesoamerican nephropathy (MeN).** In: *SALTRA.* Edited by Central American Institute for Studies on Toxic Substances (IRET-UNA) and Program on Work Environment and Health in Central America (SALTRA). Heredia, C.R.: SALTRA/IRET-UNA; 2013.
14. Weiner DE, McClean MD, Kaufman JS, Brooks DR: **The Central American epidemic of CKD**. *Clin J Am Soc Nephrol* 2013, **8**(3):504-511.
15. Torres C, Aragon A, Gonzalez M, Lopez I, Jakobsson K, Elinder CG, Lundberg I, Wesseling C: **Decreased kidney function of unknown cause in Nicaragua: a community-based survey**. *Am J Kidney Dis* 2010, **55**(3):485-496.

16. Cuadra SN, Jakobsson Kristina, Hogstedt Christer, Wesseling Catharina: **Chronic kidney disease: Assessment of current knowledge and feasibility for regional research collaboration in Central America**. In. Edited by SALTRA. Heredia, Costa Rica: SALTRA; 2006: 76.
17. Weaver VM, Fadrowski JJ, Jaar BG: **Global dimensions of chronic kidney disease of unknown etiology (CKDu): a modern era environmental and/or occupational nephropathy?** *BMC Nephrol* 2015, **16**:145.
18. Peraza S, Wesseling C, Aragon A, Leiva R, Garcia-Trabanino RA, Torres C, Jakobsson K, Elinder CG, Hogstedt C: **Decreased kidney function among agricultural workers in El Salvador**. *Am J Kidney Dis* 2012, **59**(4):531-540.
19. O'Donnell JK, Tobey M, Weiner DE, Stevens LA, Johnson S, Stringham P, Cohen B, Brooks DR: **Prevalence of and risk factors for chronic kidney disease in rural Nicaragua**. *Nephrol Dial Transplant* 2011, **26**(9):2798-2805.
20. Orantes CM, Herrera R, Almaguer M, Brizuela EG, Hernandez CE, Bayarre H, Amaya JC, Calero DJ, Orellana P, Colindres RM *et al*: **Chronic kidney disease and associated risk factors in the Bajo Lempa region of El Salvador: Nefrolempa study, 2009**. *MEDICC Review* 2011, **13**(4):14-22.
21. Raines N, Gonzalez M, Wyatt C, Kurzrok M, Pool C, Lemma T, Weiss I, Marin C, Prado V, Marcas E *et al*: **Risk factors for reduced glomerular filtration rate in a Nicaraguan community affected by Mesoamerican nephropathy**. *MEDICC Review* 2014, **16**(2):16-22.
22. Garcia-Trabanino R, Jarquin E, Wesseling C, Johnson RJ, Gonzalez-Quiroz M, Weiss I, Glaser J, Jose Vindell J, Stockfelt L, Roncal C *et al*: **Heat stress, dehydration, and kidney function in sugarcane cutters in El Salvador - A cross-shift study of workers at risk of Mesoamerican nephropathy**. *Environ Res* 2015, **142**:746-755.
23. Wesseling C, Aragon A, Gonzalez M, Weiss I, Glaser J, Bobadilla NA, Roncal-Jimenez C, Correa-Rotter R, Johnson RJ, Barregard L: **Kidney function in sugarcane cutters in Nicaragua--A longitudinal study of workers at risk of Mesoamerican nephropathy**. *Environ Res* 2016, **147**:125-132.
24. Wesseling C, Aragon A, Gonzalez M, Weiss I, Glaser J, Rivard CJ, Roncal-Jimenez C, Correa-Rotter R, Johnson RJ: **Heat stress, hydration and uric acid: a cross-sectional study in workers of three occupations in a hotspot of Mesoamerican nephropathy in Nicaragua**. *BMJ Open* 2016, **6**(12):e011034.
25. Garcia-Trabanino R, Aguilar R, Silva C. R, Mercado M. O, Merino R. L.: **End-stage renal disease among patients in a referral hospital in El Salvador**. *Rev Panam Salud Publica* 2002, **12**:202-206.
26. Laws RL, Brooks DR, Amador JJ, Weiner DE, Kaufman JS, Ramirez-Rubio O, Riefkohl A, Scammell MK, Lopez-Pilarte D, Sanchez JM *et al*: **Biomarkers of Kidney Injury Among Nicaraguan Sugarcane Workers**. *Am J Kidney Dis* 2016, **67**(2):209-217.
27. Kupferman J, Amador JJ, Lynch KE, Laws RL, Lopez-Pilarte D, Ramirez-Rubio O, Kaufman JS, Lau JL, Weiner DE, Robles NV *et al*: **Characterization of Mesoamerican Nephropathy in a Kidney Failure Hotspot in Nicaragua**. *Am J Kidney Dis* 2016, **68**(5):716-725.
28. Wijkstrom J, Gonzalez-Quiroz M, Hernandez M, Trujillo Z, Hultenby K, Ring A, Soderberg M, Aragon A, Elinder CG, Wernerson A: **Renal Morphology, Clinical Findings, and Progression Rate in Mesoamerican Nephropathy**. *Am J Kidney Dis* 2017, **69**(5):626-636.
29. Wijkstrom J, Leiva R, Elinder CG, Leiva S, Trujillo Z, Trujillo L, Soderberg M, Hultenby K, Wernerson A: **Clinical and pathological characterization of Mesoamerican nephropathy: a new kidney disease in Central America**. *Am J Kidney Dis* 2013, **62**(5):908-918.

30. Lebov JF, Valladares E, Pena R, Pena EM, Sanoff SL, Cisneros EC, Colindres RE, Morgan DR, Hogan SL: **A population-based study of prevalence and risk factors of chronic kidney disease in Leon, Nicaragua**. *Can J Kidney Health Dis* 2015, **2**:6.
31. Laux TS, Bert PJ, Barreto Ruiz GM, Gonzalez M, Unruh M, Aragon A, Torres Lacourt C: **Nicaragua revisited: evidence of lower prevalence of chronic kidney disease in a high-altitude, coffee-growing village**. *J Nephrol* 2012, **25**(4):533-540.
32. Orantes CM, Herrera R, Almaguer M, Brizuela EG, Nunez L, Alvarado NP, Fuentes EJ, Bayarre HD, Amaya JC, Calero DJ *et al*: **Epidemiology of chronic kidney disease in adults of Salvadoran agricultural communities**. *MEDICC Review* 2014, **16**(2):23-30.
33. Ordunez P, Nieto FJ, Martinez R, Soliz P, Giraldo GP, Mott SA, Hoy WE: **Chronic kidney disease mortality trends in selected Central America countries, 1997-2013: clues to an epidemic of chronic interstitial nephritis of agricultural communities**. *J Epidemiol Community Health* 2018, **72**(4):280-286.
34. Johnson RJ, Rodriguez-Iturbe B, Roncal-Jimenez C, Lanaspa MA, Ishimoto T, Nakagawa T, Correa-Rotter R, Wesseling C, Bankir L, Sanchez-Lozada LG: **Hyperosmolarity drives hypertension and CKD--water and salt revisited**. *Nat Rev Nephrol* 2014, **10**(7):415-420.
35. Robey RB: **Cyclical dehydration-induced renal injury and Mesoamerican nephropathy: as sweet by any other name?** *Kidney Int* 2014, **86**(2):226-229.
36. Johnson RJ, Stenvinkel P, Jensen T, Lanaspa MA, Roncal C, Song Z, Bankir L, Sanchez-Lozada LG: **Metabolic and Kidney Diseases in the Setting of Climate Change, Water Shortage, and Survival Factors**. *J Am Soc Nephrol* 2016, **27**(8):2247-2256.
37. Nerbass FB, Pecoits-Filho R, Clark WF, Sontrop JM, McIntyre CW, Moist L: **Occupational Heat Stress and Kidney Health: From Farms to Factories**. *Kidney International Reports* 2017, **2**(6):998-1008.
38. Lundgren K, Kuklane K, Gao C, Holmer I: **Effects of heat stress on working populations when facing climate change**. *Ind Health* 2013, **51**(1):3-15.
39. Glaser J, Lemery J, Rajagopalan B, Diaz HF, Garcia-Trabanino R, Taduri G, Madero M, Amarasinghe M, Abraham G, Anutrakulchai S *et al*: **Climate Change and the Emergent Epidemic of CKD from Heat Stress in Rural Communities: The Case for Heat Stress Nephropathy**. *Clin J Am Soc Nephrol* 2016, **11**(8):1472-1483.
40. Crowe J, Moya-Bonilla JM, Roman-Solano B, Robles-Ramirez A: **Heat exposure in sugarcane workers in Costa Rica during the non-harvest season**. *Glob Health Action* 2010, **3**.
41. Crowe J, Wesseling C, Solano BR, Umana MP, Ramirez AR, Kjellstrom T, Morales D, Nilsson M: **Heat exposure in sugarcane harvesters in Costa Rica**. *Am J Ind Med* 2013, **56**(10):1157-1164.
42. Crowe J, Nilsson M, Kjellstrom T, Wesseling C: **Heat-related symptoms in sugarcane harvesters**. *Am J Ind Med* 2015, **58**(5):541-548.
43. Paula Santos U, Zanetta DM, Terra-Filho M, Burdmann EA: **Burnt sugarcane harvesting is associated with acute renal dysfunction**. *Kidney Int* 2015, **87**(4):792-799.
44. Moyce S, Mitchell D, Armitage T, Tancredi D, Joseph J, Schenker M: **Heat strain, volume depletion and kidney function in California agricultural workers**. *Occup Environ Med* 2017, **74**(6):402-409.
45. Laws RL, Brooks DR, Amador JJ, Weiner DE, Kaufman JS, Ramirez-Rubio O, Riefkohl A, Scammell MK, Lopez-Pilarte D, Sanchez JM *et al*: **Changes in kidney function among Nicaraguan sugarcane workers**. *Int J Occup Environ Health* 2015, **21**(3):241-250.
46. Roncal-Jimenez C, Garcia-Trabanino R, Barregard L, Lanaspa MA, Wesseling C, Harra T, Aragon A, Grases F, Jarquin ER, Gonzalez MA *et al*: **Heat Stress Nephropathy From Exercise-Induced Uric Acid Crystalluria: A Perspective on Mesoamerican Nephropathy**. *Am J Kidney Dis* 2016, **67**(1):20-30.

47. Roncal-Jimenez CA, Garcia-Trabanino R, Wesseling C, Johnson RJ: **Mesoamerican Nephropathy or Global Warming Nephropathy?** *Blood Purif* 2016, **41**(1-3):135-138.
48. Roncal Jimenez CA, Ishimoto T, Lanaspa MA, Rivard CJ, Nakagawa T, Ejaz AA, Cicerchi C, Inaba S, Le M, Miyazaki M *et al*: **Fructokinase activity mediates dehydration-induced renal injury**. *Kidney Int* 2014, **86**(2):294-302.
49. Fischer RSB, Vangala C, Truong L, Mandayam S, Chavarria D, Granera Llanes OM, Fonseca Laguna MU, Guerra Baez A, Garcia F, Garcia-Trabanino R *et al*: **Early detection of acute tubulointerstitial nephritis in the genesis of Mesoamerican nephropathy**. *Kidney Int* 2018, **93**(3):681-690.
50. Kupferman J, Ramírez-Rubio O, Amador JJ, López-Pilarte D, Wilker EH, Laws RL, Sennett C, Robles NV, Lau JL, Salinas AJ *et al*: **Acute Kidney Injury in Sugarcane Workers at Risk for Mesoamerican Nephropathy**. *Am J Kidney Dis* 2018, **72**(4):475-482.
51. Crowe J. NM, Kjellstrom T, Cerdas M, Johnson R, Wesseling C.: **Repeated pre and post-shift urinalyses show kidney dysfunction among Costa Rican sugarcane cutters exposed to heat stress.** . *Occup Environ Med* 2014, **Jun;71** (Suppl 1:):A51.
52. Herath C, Jayasumana C, De Silva PMCS, De Silva PHC, Siribaddana S, De Broe ME: **Kidney Diseases in Agricultural Communities: A Case Against Heat-Stress Nephropathy**. *Kidney International Reports* 2018, **3**(2):271-280.
53. Jors E, Neupane D, London L: **Pesticide Poisonings in Low- and Middle-Income Countries**. *Environ Health Insights* 2018, **12**:1178630217750876.
54. Thundiyil JG, Stober J, Besbelli N, Pronczuk J: **Acute pesticide poisoning: a proposed classification tool**. *Bull World Health Organ* 2008, **86**(3):205-209.
55. McConnell R, Delgado-Tellez E, Cuadra R, Torres E, Keifer M, Almendarez J, Miranda J, El-Fawal HA, Wolff M, Simpson D *et al*: **Organophosphate neuropathy due to methamidophos: biochemical and neurophysiological markers**. *Arch Toxicol* 1999, **73**(6):296-300.
56. Miranda J, McConnell R, Wesseling C, Cuadra R, Delgado E, Torres E, Keifer M, Lundberg I: **Muscular strength and vibration thresholds during two years after acute poisoning with organophosphate insecticides**. *Occup Environ Med* 2004, **61**(1):e4.
57. Mostafalou S, Abdollahi M: **Pesticides: an update of human exposure and toxicity**. *Arch Toxicol* 2017, **91**(2):549-599.
58. Seneff S OLF: **Is Glyphosate a Key Factor in Mesoamerican Nephropathy?** *Journal of Environmental & Analytical Toxicology* 2018, **7**(542):11.
59. Jayasumana C, Gunatilake S, Senanayake P: **Glyphosate, hard water and nephrotoxic metals: are they the culprits behind the epidemic of chronic kidney disease of unknown etiology in Sri Lanka?** *Int J Environ Res Public Health* 2014, **11**(2):2125-2147.
60. Jayasumana C, Fonseka S, Fernando A, Jayalath K, Amarasinghe M, Siribaddana S, Gunatilake S, Paranagama P: **Phosphate fertilizer is a main source of arsenic in areas affected with chronic kidney disease of unknown etiology in Sri Lanka**. *Springerplus* 2015, **4**:90.
61. Jayasumana C, Gajanayake R, Siribaddana S: **Importance of Arsenic and pesticides in epidemic chronic kidney disease in Sri Lanka**. *BMC Nephrol* 2014, **15**:124.
62. Valcke M, Levasseur ME, Soares da Silva A, Wesseling C: **Pesticide exposures and chronic kidney disease of unknown etiology: an epidemiologic review**. *Environ Health* 2017, **16**(1):49.
63. Lebov JF, Engel LS, Richardson D, Hogan SL, Hoppin JA, Sandler DP: **Pesticide use and risk of end-stage renal disease among licensed pesticide applicators in the Agricultural Health Study**. *Occup Environ Med* 2016, **73**(1):3-12.

64. Lebov JF, Engel LS, Richardson D, Hogan SL, Sandler DP, Hoppin JA: **Pesticide exposure and end-stage renal disease risk among wives of pesticide applicators in the Agricultural Health Study**. *Environ Res* 2015, **143**(Pt A):198-210.
65. Wedin GP, Pennente CM, Sachdev SS: **Renal involvement in organophosphate poisoning**. *JAMA* 1984, **252**(11):1408.
66. Agostini M, Bianchin A: **Acute renal failure from organophospate poisoning: a case of success with haemofiltration**. *Hum Exp Toxicol* 2003, **22**(3):165-167.
67. Poovala VS, Huang H, Salahudeen AK: **Role of reactive oxygen metabolites in organophosphate-bidrin-induced renal tubular cytotoxicity**. *J Am Soc Nephrol* 1999, **10**(8):1746-1752.
68. Bloch-Shilderman E, Levy A: **Transient and reversible nephrotoxicity of sarin in rats**. *J Appl Toxicol* 2007, **27**(2):189-194.
69. Sanoff SL, Callejas L, Alonso CD, Hu Y, Colindres RE, Chin H, Morgan DR, Hogan SL: **Positive association of renal insufficiency with agriculture employment and unregulated alcohol consumption in Nicaragua**. *Ren Fail* 2010, **32**(7):766-777.
70. Tsai TL, Kuo CC, Pan WH, Chung YT, Chen CY, Wu TN, Wang SL: **The decline in kidney function with chromium exposure is exacerbated with co-exposure to lead and cadmium**. *Kidney Int* 2017, **92**(3):710-720.
71. Harari F, Sallsten G, Christensson A, Petkovic M, Hedblad B, Forsgard N, Melander O, Nilsson PM, Borné Y, Engström G *et al*: **Blood Lead Levels and Decreased Kidney Function in a Population-Based Cohort**. *Am J Kidney Dis* 2018, **72**(3):381-389.
72. Goyer RA: **Lead toxicity: a problem in environmental pathology**. *Am J Pathol* 1971, **64**(1):167-182.
73. Wallin M, Sallsten G, Lundh T, Barregard L: **Low-level cadmium exposure and effects on kidney function**. *Occup Environ Med* 2014, **71**(12):848-854.
74. Bonnell JA, Ross JH, King E: **Renal lesions in experimental cadmium poisoning**. *Br J Ind Med* 1960, **17**:69-80.
75. Hsu LI, Hsieh FI, Wang YH, Lai TS, Wu MM, Chen CJ, Chiou HY, Hsu KH: **Arsenic Exposure From Drinking Water and the Incidence of CKD in Low to Moderate Exposed Areas of Taiwan: A 14-Year Prospective Study**. *Am J Kidney Dis* 2017, **70**(6):787-797.
76. Soderland P, Lovekar S, Weiner DE, Brooks DR, Kaufman JS: **Chronic kidney disease associated with environmental toxins and exposures**. *Adv Chronic Kidney Dis* 2010, **17**(3):254-264.
77. Jha V, Prasad N: **CKD and Infectious Diseases in Asia Pacific: Challenges and Opportunities**. *Am J Kidney Dis* 2016, **68**(1):148-160.
78. Riefkohl A, Ramirez-Rubio O, Laws RL, McClean MD, Weiner DE, Kaufman JS, Galloway RL, Shadomy SV, Guerra M, Amador JJ *et al*: **Leptospira seropositivity as a risk factor for Mesoamerican Nephropathy**. *Int J Occup Environ Health* 2017:1-10.
79. Murray KO, Fischer RS, Chavarria D, Duttmann C, Garcia MN, Gorchakov R, Hotez PJ, Jiron W, Leibler JH, Lopez JE *et al*: **Mesoamerican nephropathy: a neglected tropical disease with an infectious etiology?** *Microbes Infect* 2015, **17**(10):671-675.
80. Yang CW: **Leptospirosis Renal Disease: Emerging Culprit of Chronic Kidney Disease Unknown Etiology**. *Nephron* 2017.
81. Schneider MC, Najera P, Aldighieri S, Bacallao J, Soto A, Marquino W, Altamirano L, Saenz C, Marin J, Jimenez E *et al*: **Leptospirosis outbreaks in Nicaragua: identifying critical areas and exploring drivers for evidence-based planning**. *Int J Environ Res Public Health* 2012, **9**(11):3883-3910.
82. Yang HY, Hung CC, Liu SH, Guo YG, Chen YC, Ko YC, Huang CT, Chou LF, Tian YC, Chang MY *et al*: **Overlooked Risk for Chronic Kidney Disease after Leptospiral Infection: A**

Population-Based Survey and Epidemiological Cohort Evidence. *PLoS Negl Trop Dis* 2015, **9**(10):e0004105.
83. Lee IK, Liu JW, Yang KD: **Clinical characteristics, risk factors, and outcomes in adults experiencing dengue hemorrhagic fever complicated with acute renal failure**. *Am J Trop Med Hyg* 2009, **80**(4):651-655.
84. Khalil MA, Sarwar S, Chaudry MA, Maqbool B, Khalil Z, Tan J, Yaqub S, Hussain SA: **Acute kidney injury in dengue virus infection**. *Clin Kidney J* 2012, **5**(5):390-394.
85. Mallhi TH, Khan AH, Adnan AS, Sarriff A, Khan YH, Jummaat F: **Incidence, Characteristics and Risk Factors of Acute Kidney Injury among Dengue Patients: A Retrospective Analysis**. *PLoS One* 2015, **10**(9):e0138465.
86. Mallhi TH, Khan AH, Adnan AS, Sarriff A, Khan YH, Gan SH: **Short-term renal outcomes following acute kidney injury among dengue patients: A follow-up analysis from large prospective cohort**. *PLoS One* 2018, **13**(2):e0192510.
87. Rahim Muhammad, Zaman Shahana, Mitra Palash, Jahan Ishrat, Chowdhury Tufayel, Saha Shudhanshu, Ananna Mehruba, Samad Tabassum, Hossain Mohammad, Iqbal Sarwar *et al*: **SP210 Evaluation of risk factors for acute kidney injury among patients with chikungunya: experience from a tertiary care hospital of a developing country**. *Nephrology Dialysis Transplantation* 2018, **33**((suppl_1):i414-i414).
88. Perti T, Lucero-Obusan CA, Schirmer PL, Winters MA, Holodniy M: **Chikungunya Fever Cases Identified in the Veterans Health Administration System, 2014**. *PLoS Negl Trop Dis* 2016, **10**(5):e0004630.
89. Ingrasciotta Y, Sultana J, Giorgianni F, Fontana A, Santangelo A, Tari DU, Santoro D, Arcoraci V, Perrotta M, Ibanez L *et al*: **Association of individual non-steroidal anti-inflammatory drugs and chronic kidney disease: a population-based case control study**. *PLoS One* 2015, **10**(4):e0122899.
90. Horl WH: **Nonsteroidal Anti-Inflammatory Drugs and the Kidney**. *Pharmaceuticals (Basel)* 2010, **3**(7):2291-2321.
91. Perneger TV, Whelton PK, Klag MJ: **Risk of kidney failure associated with the use of acetaminophen, aspirin, and nonsteroidal antiinflammatory drugs**. *N Engl J Med* 1994, **331**(25):1675-1679.
92. Nderitu P, Doos L, Jones PW, Davies SJ, Kadam UT: **Non-steroidal anti-inflammatory drugs and chronic kidney disease progression: a systematic review**. *Fam Pract* 2013, **30**(3):247-255.
93. Stefanovic V, Toncheva D, Polenakovic M: **Balkan nephropathy**. *Clin Nephrol* 2015, **83**(7 Suppl 1):64-69.
94. Trnacevic S, Nislic E, Trnacevic E, Tulumovic E: **Early Screening of Balkan Endemic Nephropathy**. *Mater Sociomed* 2017, **29**(3):207-210.
95. Gokmen MR, Cosyns JP, Arlt VM, Stiborova M, Phillips DH, Schmeiser HH, Simmonds MS, Cook HT, Vanherweghem JL, Nortier JL *et al*: **The epidemiology, diagnosis, and management of aristolochic acid nephropathy: a narrative review**. *Ann Intern Med* 2013, **158**(6):469-477.
96. Pfeifer H: **Revision of the North and Central American Hexandrous Species of Aristolochia (Aristolochiaceae)**. *Annals of the Missouri Botanical Garden* 1966, **53**(3):115-196.
97. Gifford FJ, Gifford RM, Eddleston M, Dhaun N: **Endemic Nephropathy Around the World**. *Kidney International Reports* 2017, **2**(2):282-292.
98. Malir F, Ostry V, Pfohl-Leszkowicz A, Malir J, Toman J: **Ochratoxin A: 50 Years of Research**. *Toxins (Basel)* 2016, **8**(7).
99. Limonciel A JP: **A Review of the Evidence that Ochratoxin A Is an Nrf2 Inhibitor: Implications for Nephrotoxicity and Renal Carcinogenicity**. *Toxins (Basel)* 2014, **6**:371-379.

100. Tao Y, Xie S, Xu F, Liu A, Wang Y, Chen D, Pan Y, Huang L, Peng D, Wang X *et al*: **Ochratoxin A: Toxicity, oxidative stress and metabolism**. *Food Chem Toxicol* 2018, **112**:320-331.
101. Correa-Rotter R, Wesseling C, Johnson RJ: **CKD of unknown origin in Central America: the case for a Mesoamerican nephropathy**. *Am J Kidney Dis* 2014, **63**(3):506-520.
102. Jayasumana C, Paranagama P, Agampodi S, Wijewardane C, Gunatilake S, Siribaddana S: **Drinking well water and occupational exposure to Herbicides is associated with chronic kidney disease, in Padavi-Sripura, Sri Lanka**. *Environ Health* 2015, **14**:6.
103. Rajapakse S, Shivanthan MC, Selvarajah M: **Chronic kidney disease of unknown etiology in Sri Lanka**. *Int J Occup Environ Health* 2016, **22**(3):259-264.
104. Ordunez P, Saenz C, Martinez R, Chapman E, Reveiz L, Becerra F: **The epidemic of chronic kidney disease in Central America**. *Lancet Glob Health* 2014, **2**(8):e440-441.
105. Ordunez P, Martinez R, Reveiz L, Chapman E, Saenz C, Soares da Silva A, Becerra F: **Chronic kidney disease epidemic in Central America: urgent public health action is needed amid causal uncertainty**. *PLoS Negl Trop Dis* 2014, **8**(8):e3019.
106. Wesseling C, van Wendel de Joode B, Crowe J, Rittner R, Sanati NA, Hogstedt C, Jakobsson K: **Mesoamerican nephropathy: geographical distribution and time trends of chronic kidney disease mortality between 1970 and 2012 in Costa Rica**. *Occup Environ Med* 2015, **72**(10):714-721.
107. Krinsky LM, Levine WJ: **An island of widows: the human face of Mesoamerican endemic nephropathy**. *Kidney Int* 2014, **86**(2):221-223.
108. Wesseling C, Crowe J, Hogstedt C, Jakobsson K, Lucas R, Wegman DH: **The epidemic of chronic kidney disease of unknown etiology in Mesoamerica: a call for interdisciplinary research and action**. *Am J Public Health* 2013, **103**(11):1927-1930.
109. Wesseling C, Crowe J, Hogstedt C, Jakobsson K, Lucas R, Wegman DH, First International Research Workshop on the Mesoamerican N: **Resolving the enigma of the mesoamerican nephropathy: a research workshop summary**. *Am J Kidney Dis* 2014, **63**(3):396-404.
110. Gonzalez-Quiroz M: **Enfermedad Renal Crónica: Prevalencia y factores de riesgo ocupacionales en el municipio de Chichigalpa**. *Tesis de postgrado.* Universidad Nacional Autónoma de Nicaragua, León; 2010.
111. Gracia-Trabanino R, Dominguez J, Jansa JM, Oliver A: **[Proteinuria and chronic renal failure in the coast of El Salvador: detection with low cost methods and associated factors]**. *Nefrologia* 2005, **25**(1):31-38.
112. Laux TS, Barnoya J, Guerrero DR, Rothstein M: **Dialysis enrollment patterns in Guatemala: evidence of the chronic kidney disease of non-traditional causes epidemic in Mesoamerica**. *BMC Nephrol* 2015, **16**:54.
113. VanDervort DR, Lopez DL, Orantes CM, Rodriguez DS: **Spatial distribution of unspecified chronic kidney disease in El Salvador by crop area cultivated and ambient temperature**. *MEDICC Review* 2014, **16**(2):31-38.
114. Gonzalez-Quiroz M, Camacho A, Faber D, Aragon A, Wesseling C, Glaser J, Le Blond J, Smeeth L, Nitsch D, Pearce N *et al*: **Rationale, description and baseline findings of a community-based prospective cohort study of kidney function amongst the young rural population of Northwest Nicaragua**. *BMC Nephrol* 2017, **18**(1):16.
115. Minnings K, Fiore M, Mosco M, Ferguson R, Leatherman S, Kerns E, Kaufman J, Fiore M, Brooks D, Amador JJ *et al*: **The Rivas Cohort Study: design and baseline characteristics of a Nicaraguan cohort**. *BMC Nephrol* 2016, **17**:93.
116. Orantes-Navarro CM, Herrera-Valdes R, Almaguer-Lopez M, Brizuela-Diaz EG, Alvarado-Ascencio NP, Fuentes-de Morales EJ: **Chronic Kidney Disease in Children and Adolescents in Salvadoran Farming Communities: NefroSalva Pediatric Study (2009-2011) (vol 18, pg 15, 2016)**. *MEDICC Review* 2016, **18**(1-2).

117. Orantes Navarro CM, Herrera Valdes R, Lopez MA, Calero DJ, Fuentes de Morales J, Alvarado Ascencio NP, Vela Parada XF, Zelaya Quezada SM, Granados Castro DV, Orellana de Figueroa P: **Epidemiological characteristics of chronic kidney disease of non-traditional causes in women of agricultural communities of El Salvador**. *Clin Nephrol* 2015, **83**(7 Suppl 1):24-31.
118. Vela XF, Henriquez DO, Zelaya SM, Granados DV, Hernandez MX, Orantes CM: **Chronic kidney disease and associated risk factors in two Salvadoran farming communities, 2012**. *MEDICC Review* 2014, **16**(2):55-60.
119. Lopez-Marin L, Chavez Y, Garcia XA, Flores WM, Garcia YM, Herrera R, Almaguer M, Orantes CM, Calero D, Bayarre HD *et al*: **Histopathology of chronic kidney disease of unknown etiology in Salvadoran agricultural communities**. *MEDICC Review* 2014, **16**(2):49-54.
120. Lunyera J, Mohottige D, von Isenburg M, Jeuland M, Patel UD, Stanifer JW: **CKD of uncertain etiology: A systematic review**. *Clin J Am Soc Nephrol* 2016, **11**(3):379-385.
121. Said S, Hernandez GT: **Environmental Exposures, Socioeconomics, Disparities, and the Kidneys**. *Adv Chronic Kidney Dis* 2015, **22**(1):39-45.
122. Gonzalez-Quiroz M, Pearce N, Caplin B, Nitsch D: **What do epidemiological studies tell us about chronic kidney disease of undetermined cause in Meso-America? A systematic review and meta-analysis**. *Clin Kidney J* 2018, **11**(4):496-506.
123. Iwagami M, Tomlinson LA, Mansfield KE, Casula A, Caskey FJ, Aitken G, Fraser SDS, Roderick PJ, Nitsch D: **Validity of estimated prevalence of decreased kidney function and renal replacement therapy from primary care electronic health records compared with national survey and registry data in the United Kingdom**. *Nephrol Dial Transplant* 2017, **32**(suppl_2):ii142-ii150.
124. Gonzalez-Quiroz M, Smpokou ET, Silverwood RJ, Camacho A, Faber D, Garcia BR, Oomatia A, Hill M, Glaser J, Le Blond J *et al*: **Decline in Kidney Function among Apparently Healthy Young Adults at Risk of Mesoamerican Nephropathy**. *J Am Soc Nephrol* 2018, **29**(8):2200-2212.
125. Caplin Ben, González-Quiroz Marvin, Pearce Neil: **Gaining insights into the evolution of CKDnt from community-based follow up studies**. In: *Second International Workshop on Mesoamerican Nephropathy.* Edited by SALTRA, vol. Volume 2. San José: SALTRA; 2015.
126. Genovese G, Friedman DJ, Ross MD, Lecordier L, Uzureau P, Freedman BI, Bowden DW, Langefeld CD, Oleksyk TK, Uscinski Knob AL *et al*: **Association of trypanolytic ApoL1 variants with kidney disease in African Americans**. *Science* 2010, **329**(5993):841-845.
127. Limou S, Vince N, Parsa A: **Lessons from CKD-Related Genetic Association Studies-Moving Forward**. *Clin J Am Soc Nephrol* 2018, **13**(1):140-152.
128. Noone DG, Iijima K, Parekh R: **Idiopathic nephrotic syndrome in children**. *Lancet* 2018, **392**(10141):61-74.
129. Perez-Gomez MV, Martin-Cleary C, Fernandez-Fernandez B, Ortiz A: **Meso-American nephropathy: what we have learned about the potential genetic influence on chronic kidney disease development**. *Clin Kidney J* 2018, **11**(4):491-495.
130. Bodin T, Garcia-Trabanino R, Weiss I, Jarquin E, Glaser J, Jakobsson K, Lucas RA, Wesseling C, Hogstedt C, Wegman DH *et al*: **Intervention to reduce heat stress and improve efficiency among sugarcane workers in El Salvador: Phase 1**. *Occup Environ Med* 2016, **73**(6):409-416.
131. Quinteros E, Ribo A, Mejia R, Lopez A, Belteton W, Comandari A, Orantes CM, Pleites EB, Hernandez CE, Lopez DL: **Heavy metals and pesticide exposure from agricultural activities and former agrochemical factory in a Salvadoran rural community**. *Environ Sci Pollut Res Int* 2017, **24**(2):1662-1676.

132. Ramirez-Rubio O, Brooks DR, Amador JJ, Kaufman JS, Weiner DE, Scammell MK: **Chronic kidney disease in Nicaragua: a qualitative analysis of semi-structured interviews with physicians and pharmacists**. *BMC Public Health* 2013, **13**:350.
133. Solis-Zepeda G: **Impacto de las medidas preventivas para evitar el deterioro de la función renal por el Síndrome de Golpe por Calor en trabajadores agrícolas del Ingenio San Antonio del Occidente de Nicaragua, Ciclo Agrícola 2004-2005.** Faculty of Medical Sciences: National Autonomous University of Nicaragua, Leon; 2007.
134. Junglee NA, Di Felice U, Dolci A, Fortes MB, Jibani MM, Lemmey AB, Walsh NP, Macdonald JH: **Exercising in a hot environment with muscle damage: effects on acute kidney injury biomarkers and kidney function**. *Am J Physiol Renal Physiol* 2013, **305**(6):F813-820.
135. Wegman DH, Apelqvist J, Bottai M, Ekstrom U, Garcia-Trabanino R, Glaser J, Hogstedt C, Jakobsson K, Jarquin E, Lucas RAI *et al*: **Intervention to diminish dehydration and kidney damage among sugarcane workers**. *Scand J Work Environ Health* 2018, **44**(1):16-24.
136. Acharya P, Boggess B, Zhang K: **Assessing Heat Stress and Health among Construction Workers in a Changing Climate: A Review**. *Int J Environ Res Public Health* 2018, **15**(2).
137. Donoghue AM, Bates GP: **The risk of heat exhaustion at a deep underground metalliferous mine in relation to surface temperatures**. *Occup Med (Lond)* 2000, **50**(5):334-336.
138. Donoghue AM, Sinclair MJ, Bates GP: **Heat exhaustion in a deep underground metalliferous mine**. *Occup Environ Med* 2000, **57**(3):165-174.
139. Krishnamurthy M, Ramalingam P, Perumal K, Kamalakannan LP, Chinnadurai J, Shanmugam R, Srinivasan K, Venugopal V: **Occupational Heat Stress Impacts on Health and Productivity in a Steel Industry in Southern India**. *Saf Health Work* 2017, **8**(1):99-104.
140. Lundgren K, Kuklane K, Venugopal V: **Occupational heat stress and associated productivity loss estimation using the PHS model (ISO 7933): a case study from workplaces in Chennai, India**. *Global Health Action* 2014, **7**:10.3402/gha.v3407.25283.
141. Smpokou Evangelia-Theano, González-Quiroz Marvin, Martins Carla, Alvito Paula, Le Blond Jennifer, Glaser Jason, Aragon Aurora, Wesseling Catharina, Nitsch Dorothea, Pierce Neil *et al*: **Systematic investigation of environmental exposures in young adults with declining kidney function in a population at risk of Mesoamerican Nephropathy (MeN)** In.; 2018: 30.
142. Ghosh R, Siddarth M, Singh N, Tyagi V, Kare PK, Banerjee BD, Kalra OP, Tripathi AK: **Organochlorine pesticide level in patients with chronic kidney disease of unknown etiology and its association with renal function**. *Environ Health Prev Med* 2017, **22**(1):49.
143. Instituto Nacional de Información para el Desarrollo (INIDE): **VIII Censo de Población y IV de Vivienda**. In: *Censo Nacional.* Edited by INIDE, IV edn. Managua: INIDE; 2005.
144. Pena R, Perez W, Melendez M, Kallestal C, Persson LA: **The Nicaraguan Health and Demographic Surveillance Site, HDSS-Leon: a platform for public health research**. *Scand J Public Health* 2008, **36**(3):318-325.
145. Kjellstrom T, Crowe J: **Climate change, workplace heat exposure, and occupational health and productivity in Central America**. *Int J Occup Environ Health* 2011, **17**(3):270-281.
146. Lundgren-Kownacki K, Kjellberg SM, Gooch P, Dabaieh M, Anandh L, Venugopal V: **Climate change-induced heat risks for migrant populations working at brick kilns in India: a transdisciplinary approach**. *Int J Biometeorol* 2018, **62**(3):347-358.
147. Venugopal V, Chinnadurai JS, Lucas RA, Kjellstrom T: **Occupational Heat Stress Profiles in Selected Workplaces in India**. *Int J Environ Res Public Health* 2015, **13**(1).

148. Budd GM: **Wet-bulb globe temperature (WBGT)--its history and its limitations**. *J Sci Med Sport* 2008, **11**(1):20-32.
149. Cook EL: **Epidemiological approach to heat trauma**. *Mil Med* 1955, **116**(5):317-322.
150. Epstein Y, Moran DS: **Thermal comfort and the heat stress indices**. *Ind Health* 2006, **44**(3):388-398.
151. Lucas. RA, Bodin. T, García-Trabanino. R, Wesseling. C, Glaser. J, Weiss. I, Jarquin. E, Jakobsson. K, Wegman. DH: **Heat stress and workload associated with sugarcane cutting - an excessively strenuous occupation!** *Extrem Physiol Med* 2015, **4**((Suppl 1)):A23.
152. Tawatsupa B, Lim LL, Kjellstrom T, Seubsman SA, Sleigh A, Thai Cohort Study T: **Association between occupational heat stress and kidney disease among 37,816 workers in the Thai Cohort Study (TCS)**. *J Epidemiol* 2012, **22**(3):251-260.
153. Sundstrup E, Hansen AM, Mortensen EL, Poulsen OM, Clausen T, Rugulies R, Moller A, Andersen LL: **Retrospectively assessed physical work environment during working life and risk of sickness absence and labour market exit among older workers**. *Occup Environ Med* 2018, **75**(2):114-123.
154. Tawatsupa B, Yiengprugsawan V, Kjellstrom T, Seubsman SA, Sleigh A, Thai Cohort Study T: **Heat stress, health and well-being: findings from a large national cohort of Thai adults**. *BMJ Open* 2012, **2**(6).
155. Chan AP, Yang Y: **Practical on-site measurement of heat strain with the use of a perceptual strain index**. *Int Arch Occup Environ Health* 2016, **89**(2):299-306.
156. Departmen of the Army and Air Force: **Technical bulletin: Heat stress control and heat causalty management (TB MED 507) Air Force Pamphlet 48-152(1).** In. Edited by Departmen of the Army and Air Force, vol. 1. Washington, DC.: Departmen of the Army and Air Force,; 2003: 72.
157. Shlipak MG, Matsushita K, Arnlov J, Inker LA, Katz R, Polkinghorne KR, Rothenbacher D, Sarnak MJ, Astor BC, Coresh J *et al*: **Cystatin C versus creatinine in determining risk based on kidney function**. *N Engl J Med* 2013, **369**(10):932-943.
158. Urbschat A, Obermuller N, Haferkamp A: **Biomarkers of kidney injury**. *Biomarkers* 2011, **16 Suppl 1**:S22-30.
159. Nitsch D, Sandling JK, Byberg L, Larsson A, Tuvemo T, Syvanen AC, Koupil I, Leon DA: **Fetal, developmental, and parental influences on cystatin C in childhood: the Uppsala Family Study**. *Am J Kidney Dis* 2011, **57**(6):863-872.
160. Garcia-Trabanino R, Trujillo Z, Colorado AV, Magana Mercado S, Henriquez CA, En nombre de la Asociacion de Nefrologia e Hipertension Arterial de El S: **Prevalence of patients receiving renal replacement therapy in El Salvador in 2014**. *Nefrologia* 2016, **36**(6):631-636.
161. Gonzalez-Bedata C, Rosa-Diez, G., Ferreiro, A.: **Latin American Dialysis and Renal Transplantation Registry: Theimportance of the development of national registries in Latin America**. *Nefrología Latinoamericana* 2017, **14**(1):12-21.
162. Compliance Advisor Ombudsman: **Medical needs assessment of the Chichigalpa community health center and dialysis options for chronic renal insufficiency patients**. In: *Independent Consultant Report to the Office of the Compliance Advisor/Ombudsman of the International Finance Corporation and Multilateral Investment Guarantee Agency.* Compliance Advisor Ombudsman; 2011: 48.
163. Instituto Nicaragüense de Seguridad Social (INSS): **Anuario estadístico 2017**. In. Edited by División General de Estudios Económico DdEE. Managua: INSS; 2017: 384.
164. **Nicaraguan Sugar Estates Limited (SER San Antonio). Sugarcane harvest. 2017** [http://www.nicaraguasugar.com/index.php?option=com_content&view=article&id=9&Itemid=176]

Appendix A: Ethical approvals

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Observational / Interventions Research Ethics Committee

Mr Marvin Gonzaelz
LSHTM

25 September 2017

Dear Mr Marvin Gonzaelz

Study Title: Occupational kidney disease among young populations in Northwest Nicaragua

LSHTM Ethics Ref: 14363

Thank you for responding to the Observational Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Protocol / Proposal	Research project-Marvin Gonzalez	15/06/2017	Final
Investigator CV	Resume 2017	21/06/2017	doc
Information Sheet	Research consent form community cohort	01/07/2017	1.1
Local Approval	IRB approval_followup biopsy	01/07/2017	1.1
Local Approval	Karolinska IRB approval 1.1	01/07/2017	1.1
Local Approval	Karolinska IRB approval 1	01/07/2017	1.1
Local Approval	LSHTM IRB	01/07/2017	1.1
Local Approval	UNAN Leon IRB approval biopsy	01/07/2017	1.1
Local Approval	UNAN-León IRB approval	01/07/2017	1.1
Information Sheet	Kidney biopsy informed consent	01/07/2017	1.1
Covering Letter	Clarification letter for IRB	12/09/2017	1

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

An annual report should be submitted to the committee using an Annual Report form on the anniversary of the approval of the study during the lifetime of the study.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

[redacted]

Professor John DH Porter
Chair

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

Improving health worldwide

Universidad Nacional Autónoma de Nicaragua
Facultad de Ciencias Médicas
UNAN - León

Comité de Ética para Investigaciones Biomédicas (CEIB)
"Dr. Uriel Guevara Guerrero"
FWA00004523 / IRB00003342

León, 05 septiembre del 2014

ACTA No.116

-Profesor Neil Pearce
-Dr. Ben Caplin
Investigadores

Estimados Académicos:

El CEIB les informa que ha recibido el trabajo de Investigación, para que sea evaluado por este Comité, titulado: **"Enfermedad renal de origen ocupacional en la Costa del Pacífico de América Central"**, *después de examinar cuidadosamente dicho Protocolo tanto en su Diseño como en sus aspectos éticos y que se adapta a la Declaración de Helsinki y a la Ley General de Salud vigente del país, concluye que pueden darle continuidad.*

Como Comité de Ética, valoramos muy positivamente la importancia de este trabajo sobre este tema. Copia de esta carta debe estar presente en el Protocolo e informe final.

Sin otro particular, nos es grato suscribirnos.

Atentamente,

[redacted]
DRA. [redacted] S.
Presidenta del CEIB
Facultad de CC. MM.

DR. ORLANDO MORALES N.
Secretario del CEIB
Facultad de CC, MM.

[redacted]
DRA. MERCEDES CACERES, PHD
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Cc: Archivo
NAPS/rhl

Por la Pertinencia y Excelencia Académica

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Expiration data
31/08/2015

Appendix B: Questionnaire

RESEARCH

BASELINE QUESTIONNAIRE (O_CKD_PC)

Questionnaire code _____ **Community code** _____

Date __/__/___ Hour: _____

Good morning, my name is _____, I work at the National Autonomous University of Nicaragua, León. We are collaborating with London School of Hygiene and Tropical Medicine and University College London in a research Project of Chronic Kidney Disease in North-western Nicaragua.

Before starting with the questionnaire, we would such as to ask you if are you agree to be part of this study? If you agree to enrol in this study, please sign the inform consent.

Anthropometrics measurements

Weight ☐☐.☐ kg Height ☐.☐☐ meter

Blood pressure: ☐☐☐ / ☐☐☐ mm/Hg (Systolic/diastolic)

Samples (mark with a X if you took it) Blood____ Urine:_____ Water_____

You will now be asked questions about different aspects related to your life and work.

DEMOGRAPHICS	
1) Age ☐☐ *year old*	**2) Sex** 1. Women ☐ 2. Men ☐
3) Last year of school approved *(Write the last grade or year approved)* **Total of schooling years: _____**	☐☐ 1.Primary ☐☐ 2. Secondary ☐☐ 3. Polytechnic ☐☐ 4. University
SOCIOECONOMIC CONDITIONS	
4) How many of your family members living in the same house are currently working?	☐☐

5) How much do you earn?	C$ ☐☐☐☐☐ monthly,
6) How much is your family income per month? **(**Sum the entry in Córdobas of ALL family members and remittances from abroad**)**	Salaries = C$ ☐☐☐☐☐ Family remittances = C$ ☐☐☐☐☐ Other income = C$ ☐☐☐☐☐ Total = C$ ☐☐☐☐☐
7) How many people depend on that income?	Children ☐☐ + Adults ☐☐ Total= ☐☐
8) Where does the water you drink come from? *(check more than one options)*	Well ☐ River ☐ Waterhole ☐ Pipped water ☐ (if it is well, fill the additional questions) Perforated: 1. Yes ☐ 2. No ☐ Excavated: 1. Yes ☐ 2. No ☐ Protected: 1. Yes ☐ 2. No ☐ Agricultural plantations near the water source: 1. Yes ☐ (Consider crops close at 100 mts) 2. No ☐ Type of crops: _____
9) Do you treat the drinking water? (Boiling, chlorine, etc.)	1. Boiling ☐ 2. Chlorine ☐ 3. Home filters ☐ 4. Other ☐ _____ 5. None ☐
10) Where do you defecate (take a dump or have a dump?	1. Latrine ☐ 2. Toilet ☐ 3. Outdoor ☐
CURRENT OCCUPATION	
11) What is your current job? (Agriculture, sugarcane cutter, farmer, water applicator construction, etc)	_____
12) What task do you perform?	

13) How many years have you been working in your current job?	☐☐ years ☐☐ months
14) How many months in a year do you work?	☐☐ months
15) How many hours do you work daily?	☐☐☐hours
16) Do you work on your own or for someone else?	1.On my own ☐ 2.For someone ☐
17) Are you affiliated to the national social security system?	1. Yes ☐ 2.No ☐
18) What age do you had when you started working for the first time?	_____ Year old

OCCUPATIONS HISTORY

19) Since you started working, what have you worked for?
(not include those jobs with less than 3 months. Begin with the first and finish the last job (no include the current job). Include the unemployed period, please

Age	Occupation *(example: agricultural worker, construction worker, Vendor, etc)*	What task did you perform? *(Example: seeder, Construction, apply pesticide, etc.)*	How long have you been working *(years)*	How many days did you work per week?	How many hours did you work per day?

HEAT EXPOSURES

20) Your work is carried out mostly?:	1. Indoors. 2. Mostly indoors. 3. Outdoors. 4. Mostly outdoors

	5. Other, specify: _____
21) Do you work in a very hot working environment?	1. Seldom or never. 2. Few times. 3. Regularly. 4. Frequently. 5. Always or almost always.
22) If it is regularly or more often: ¿Do you have possibilities to cool off when you needed?	1.No ☐ 2. Yes ☐ specify: _____
23) Do you have breaks during your workday?	1. Yes ☐ 2.No ☐
24) What is the total duration of your breaks? (no including the lunch break, please)	☐☐☐ minutes
25) How often do you take breaks?	☐☐ times
26) How long do you take for your lunch time?	☐☐☐ minutes
27) Is there shade available during breaks in your workplace?	1.Yes ☐ 2.No ☐
28) Do you work at a high work speed?	1. Yes ☐ 2.No ☐
29) If it is yes: Do you have possibility to slow down when needed?	1. Yes ☐ 2.No ☐
30) If it is not, explain why?	_____
31) How long time do you take to commute to your workplace?	☐☐☐ minutes
32) What is your means of transportation to the workplace?	☐ 1. Bike ☐ 2. On foot ☐ 3. Open truck, sitting. ☐ 4. Open truck, standing ☐ 5. Bus. ☐ 6. Other: _____
33) When you have arrived at work, are you already get sweating heavily?	1.Yes ☐ 2.No ☐

34) Do you push, throw or lift heavy objects or equipment?	1.Yes ☐ 2.No ☐		
35) If handling heavy loads, what is the approximate weight(s) of the objects or equipment?	☐ 1. Up to 25 lbs ☐ 2. Between 26 y 50 lbs ☐ 3. Between 51 y 100 lbs ☐ 4. More than 100 lbs		
36) How much physical effort did you exert last week at work?	☐ 1. Slight effort ☐ 2. Moderate effort ☐ 3. Hard effort ☐ 4. Very hard effort ☐ 5. Did not work last week		
37) Have you worked on a cotton plantation?	1.Yes ☐ 2.No ☐ (If it is no go to the question 38)		
If it is yes	**How long have you worked?**	**What task did you perform?**	
38) Do or have you worked in a banana plantation?	1.Yes ☐ 2.No ☐ (if it is no go to question 39)		
If yes	**How long do or have you work(ed)?**	**What task do or did you perform?**	
39) Do you work or have you been working in a banana packaging plant?	1.Yes ☐ ☐☐ Years 2.No ☐(If it is no go to question 40)		
40) If you are not currently working in sugarcane, have you worked in sugarcane?	1.Yes ☐ 2.No ☐ (if it is no go to question 41)		
If it is yes	**How many years have you worked?**	**How many months have you worked in a year?**	**What kind of tasks have you performed?**

HYDRATATION HABITS ***(calculate in litres always, for*** *expample. 1 glass = 0.250 L*

Could you tell me about the drinks that you drank yesterday, if you drank and how much you drank since you woke up?

On waking

41) Did you drink something?	**42) What did you drink?**	**43) How much did you drink?**
1.Yes ☐ 2.No ☐	_____ _____ _____ _____ _____ _____	_____ **Litres** _____ **Litres** _____ **Litres**

During the morning

44) Did you drink something?	**45) What did you drink?**	**46) How much did you drink?**
1.Yes ☐ 2.No ☐	_____ _____ _____ _____ _____ _____	_____ **Litres** _____ **Litres** _____ **Litres**

At noon

47) Did you drink something?	**48) What did you drink?**	**49) How much did you drink?**
1.Yes ☐ 2.No ☐	_____ _____ _____ _____ _____ _____	_____ **Litres** _____ **Litres** _____ **Litres**

Afternoon

50) Did you drink something?	**51) What did you drink?**	**52) How much did you drink?**
1.Yes ☐ 2.No ☐	_____ _____	_____ **Litres**

	_____ _____ _____ _____	_____ **Litres** _____ **Litres**
During dinner		
53) Did you drink something?	**54) What did you drink?**	**55) How much did you drink?**
1.Yes ☐ 2.No ☐	_____ _____ _____ _____ _____ _____	_____ **Litres** _____ **Litres** _____ **Litres**
After dinner		
56) Did you drink something?	**57) What did you drink?**	**58) How much did you drink?**
1.Yes ☐ 2.No ☐	_____ _____ _____ _____ _____ _____	_____ **Litres** _____ **Litres** _____ **Litres**
If the interviewee is a worker:		
59) Did you go to work yesterday?	1.Yes ☐ 2.No ☐	
60) If not, When you work do you drink more fluids?	1. Much more ☐ 2. More ☐ 3. The same ☐ 4. Less ☐	
OTHER HABITS		
61) Do you currently smoke?	1.Yes ☐ 2.No ☐ *(if it is no go to question 64)*	
62) How many cigarette do you smoke a day?	☐☐ cigarette/day	
63) What age did you start of smoking?	☐☐ Years	
64) if you do not currently smoke: Did you smoke before?	1.Yes ☐ 2.No ☐ *(if it is no go to question 69)*	
65) How many cigarettes did you smoke a day?	☐☐	

66) What age did you start of smoking and at what age did you stop of smoking?	☐☐Age at start. ☐☐Age at finish
67) If you smoke intermittently, how many years have you smoked?	_____ Years smoked
68) Do you currently drink alcohol?	1.Yes ☐ 2.No ☐ *(if it is no go to question 70)*

69) What kind of alcohol do you drink? *(Include all kind alcohol beverage that the interviewee drinks)*

Type	**Quantity**	**Frequency** *(Daily, Weekly, Monthly, etc.)*	**Years of drinking this type of alcohol**
Beer ☐	☐☐ bottles 12 ounces (1 liter = 3 bottles)		☐☐
Rum ☐	☐☐ Shoot (1 small bottle = 13 shoots)		☐☐
Caballito/Perla/Ron plata ☐	☐☐ shoot (1 small bottle = 13 shoots)		☐☐
Other ☐ (Wine, vodka, tequila, etc.)	☐☐ _____		☐☐

70) If you do not drink now, did you drink before?	☐ 1.Yes ☐2.No *(If it is no go to question 71)*

Type	**Quantity**	**Frequency** *(daily, Weekly, Monthly, etc.)*	**Years of drinking this type of alcohol**
Beer ☐	☐☐ bottles 12 ounces (1 liter = 3 bottles)		☐☐
Rum ☐	☐☐ Shoot (1 small bottle = 13 shoots)		☐☐
Caballito/Perla/Ron plata/ ☐	☐☐ shoot (1 small bottle = 13 shoots)		☐☐
Other ☐ ____ _____ (Wine, vodka, tequila, etc.)	☐☐ _____		☐☐

71) Do you take or have you taken illegal drugs?	☐1.Yes ☐ 2.No *(If it is no, go to question 73)*

72)	**Answers the following questions**	**1. Have you ever tried in your life?**	**2. Have you smoked in the last year?**	**3. Have you smoked in the last 30 days?**
	1. Marijuana	1.Yes 2.No	1.Yes 2.No	1.Yes 2.No

	2. Floripon (angel´s trumpet)	1. Yes	2.No	1.Yes	2.No	1.Yes	2.No
	3. Mushrooms	1.Yes	2.No	1.Yes	2.No	1.Yes	2.No
	4. Cocaine	1.Yes	2.No	1.Yes	2.No	1.Yes	2.No
	5. Crack	1.Yes	2.No	1.Yes	2.No	1.Yes	2.No
	6. Glue	1.Yes	2.No	1.Yes	2.No	1.Yes	2.No
	7. Other drugs (specify):	1.Yes	2.No	1.Yes	2.No	1.Yes	2.No

HEALTH AND WELFARE

73) How do you consider your health status?

☐ 1. Very good ☐ 2.Good ☐ 3. Regular ☐ 4.Bad ☐ 5. Very bad

74) How often do you do exercise?

☐ *1. Never* ☐ *2.Occasionally* ☐ *3. 2 to 3 times x week* ☐ *4. 4 to 5 times x week*

☐ *5. Every days*

75) How often do you eat fruits, vegetables and salads?

☐ *1. Never* ☐ *2.Occasionally* ☐ *3. 2 to 3 times x week* ☐ *4. 4 to 5 times x week*

☐ *5. Every days*

76) ¿Do you eat your food without salt or with very Little salt?

☐ *1. Always* ☐ *2. Most of the time* ☐ *3.Few times* ☐ *4.Never* ☐ *5. Add salt to my food*

77) In the last 4 weeks have you felt?

a) Back pain?

☐ 1. Cervical ☐ 2. Thoracic ☐ 3. Lumbosacral ☐ 4. Has not felt anything

b) Arm or leg pain?

☐ 1. Shoulders ☐ 2.Elbows ☐ 3. Wrists ☐ 4. Hands ☐ 5. Knee ☐ 6.Ankles ☐ 7. Feet ☐ 8. Other

78) In the last 12 months of work, have you suffered any injuries (injury, fracture, etc) due to an accident at work?	☐ 1. Yes ☐ 2.No **¿Specify?** _____

Nephrotoxic medications (show the catalogue)

79) Have you taken any of these pain medications that you see in the catalogue?	☐1.Yes ☐ 2.No
80) Ibuprofen, Diclofenac,	☐0. Never

	☐ 1.Only occasionally ☐ 2. Regularly or intermittently ☐1.For 1 month or more (___months) ☐2.Less than a month (___weeks) ☐ 3.Daily ☐1.For a week or more (___week) ☐2.Less than a week (___days)
81) Aspirin	☐0. Never ☐ 1.Only occasionally ☐ 2. Regularly or intermittently ☐1.For 1 month or more (___months) ☐2.Less than a month (___weeks) ☐ 3.Daily ☐1.For a week or more (___week) ☐2.Less than a week (___days)
82) Paracetamol (acetaminofen)	☐0. Never ☐ 1.Only occasionally ☐ 2. Regularly or intermittently ☐1.For 1 month or more (___months) ☐2.Less than a month (___weeks) ☐ 3.Daily ☐1.For a week or more (___week) ☐2.Less than a week (___days)
83) Could you tell me where was the pain?	_____
84) Have you received antibiotics for injection more than a week? **Show catalogue** (gentamicin, amikacin)	☐0.Never ☐ 1.Only occasionally ☐ 2. Regularly or intermittently

	☐ 3.Daily
85) Could you tell me for what kind of infection?	_____
86) Do you take or have you taken Furosemide, phenazopyridine? **Show catalogue**	☐0.Never ☐ 1.Only occasionally ☐ 2. Regularly or intermittently ☐ 3.Daily
87) Do you drink a medication for high blood pressure?	☐1.Yes ☐ 2.No

FAMILY BACKGROUND

88) Has a family member been diagnosed with CKD (Currently or before)	**1.** Father _____ **2.** Mother _____ **3.** Brothers (# _____) **4.** Sisters (# _____) **5.** Cousins (# _____) **6.** Nephews (# ____) **7.** Nieces (# _____) **8.** Uncles (#_____) **9.** None	**89) Has he worked in agriculture?** ☐1.Yes ☐2.No **a)** Father _____ **b)** Mother _____ **c)** Brothers (# _____) **d)** Sisters (# _____) **e)** Cousins (# _____) **f)** Nephews (# _____) **g)** Nieces (# _____) **h)** Uncles (#_____) **90) Has he worked in sugarcane?** ☐1.Yes ☐2.No **a)** Father _____ **b)** Mother _____ **c)** Brothers (# _____) **d)** Sisters (# _____) **e)** Cousins (# _____) **f)** Nephews (# _____) **g)** Nieces (# _____) **h)** Uncles (#_____)

PERSONAL HEALTH HISTORY

91) Have you been diagnosed with any of the following diseases: (confirmed	a) High blood pressure: 1.Yes ☐ 2.No ☐ b) Diabetes: 1.Yes ☐ 2.No ☐

by a physician and under treatment)?	c) Nephrolithiasis 1.Yes ☐ 2.No ☐ d) Arthritis 1.Yes ☐ 2.No ☐ e) Other 1.Yes ☐ 2.No ☐ Specify_____
92) Have you been medically diagnosed with urinary tract infection?	☐1.Yes ☐ 2.No *(if it is no go to question 94)*
93) If it is yes did they laboratory test the urine?	☐1.Yes ☐ 2.No
94) How many times have you had urinary tract infections in the last year?	☐☐
95) How long ago was the last time you had a urinary tract infection?	☐☐ months
96) Do you have a spouse or partner?	☐1.Yes ☐ 2.No
97) If it is yes, do you or your partner intend to get pregnant?	☐1.Yes ☐ 2.No
98) How long have you been trying to get got pregnant?	☐☐ months
99) How many children do you have?	Still births ☐☐ Abortions ☐☐
100) Did you or your wife get pregnant easily?	☐1.Yes ☐ 2.No
101) How long ago was you got the last pregnancy?	☐☐ months
102) Have you used birth control?	☐1.Yes ☐ 2.No
103) Have you been diagnosed with infertility?	☐1.Yes ☐ 2.No

104) Have you had premature birth?	☐1.Yes ☐ 2.No
105) If it is yes, how premature were they?	☐☐ weeks
106) Have you had a child who was small at birth?	☐1.Yes ☐ 2.No
HEAT STRESS AND DEHYDRATION SYMPTOMS	
107) Have you fainted or passed out due to heat?	☐1.Yes ☐ 2.No *(If it is yes, go to question 110)*
108) If it is yes, has it been on your job?	☐1.Yes ☐ 2.No
109) If it is yes, what task were you doing?	_____
110) Have you lost weight in the last 6 months?	☐1.Yes ☐ 2.No *(if it is yes, go to question 114)*
111) If it is yes, in what circumstance?	1. Working, Specify_____ 2. Doing exercise or sport 3. Other, specify_____
112) How many pounds have you lost in the last 6 months?	☐☐ lbs
113) What frame time have you lost that weight?	**1.** _____ days **2.** _____ weeks **3.** _____months
114) Could you tell me if you have seen the aristolochia plant or the flower that I show in the catalogue)?	☐1.Yes ☐ 2.No
115) If it is yes, where have you seen it grow?	_____
116) Does it grow in sugarcane fields?	☐1.Yes ☐ 2.No ☐ 9.Do not know

Have you experienced any of the following symptoms in the last 6 months?

Symptoms		**How often** (check with a circle)
1) Extremely dry mouth	☐1.Yes Today? Yes ___ No___ ☐ 2.No	1. Almost every day or every day 2. At least once a week 3. Several times a month 4. Once during these months
2) Burning sensation while urinating or Chistata	☐1.Yes Today? Yes ___ No___ ☐ 2.No	1. Almost every day or every day 2. At least once a week 3. Several times a month 4. Once during these months
3) Very little urine	☐1.Yes Today? Yes ___ No___ ☐ 2.No	1. Almost every day or every day 2. At least once a week 3. Several times a month 4. Once during these months
4) Very dark urine	☐1.Yes Today? Yes ___ No___ ☐ 2.No	1. Almost every day or every day 2. At least once a week 3. Several times a month 4. Once during these months
5) Cramps	☐1.Yes Today? Yes ___ No___ ☐ 2.No	1. Almost every day or every day 2. At least once a week 3. Several times a month 4. Once during these months
6) Headache	☐1.Yes Today? Yes __ No___ ☐ 2.No	1. Almost every day or every day 2. At least once a week 3. Several times a month 4. Once during these months
7) Palpitations (feeling your heart is beating very fast)	☐1.Yes Today? Yes ___ No___ ☐ 2.No	1. Almost every day or every day 2. At least once a week 3. Several times a month 4. Once during these months

Symptoms		How often (check with a circle)
8) Fever	☐1.Yes Today? Yes ___ No___ ☐ 2.No	1. Almost every day or every day 2. At least once a week 3. Several times a month 4. Once during these months
9) Muscle weakness	☐1.Yes Today? Yes ___ No___ ☐ 2.No	1. Almost every day or every day 2. At least once a week 3. Several times a month 4. Once during these months
10) Inflammation of hands or feet	☐1.Yes Today? Yes ___ No___ ☐ 2.No	1. Almost every day or every day 2. At least once a week 3. Several times a month 4. Once during these months
11) Nausea	☐1.Yes Today? Yes ___ No___ ☐ 2.No	1. Almost every day or every day 2. At least once a week 3. Several times a month 4. Once during these months
12) Rapid breathing or difficulty breathing	☐1.Yes Today? Yes ___ No___ ☐ 2.No	1. Almost every day or every day 2. At least once a week 3. Several times a month 4. Once during these months
13) Dizziness	☐1.Yes Today? Yes ___ No___ ☐ 2.No	1. Almost every day or every day 2. At least once a week 3. Several times a month 4. Once during these months
14) Fainting, passing out	☐1.Yes Today? Yes ___ No___ ☐ 2.No	1. Almost every day or every day 2. At least once a week 3. Several times a month 4. Once during these months
15) Diarrhoea	☐1.Yes Today? Yes ___ No___	1. Almost every day or every day 2. At least once a week

Symptoms		How often (check with a circle)
	☐ 2.No	3. Several times a month 4. Once during these months
16) Vomiting	☐1.Yes Today? Yes ___ No___ ☐ 2.No	1. Almost every day or every day 2. At least once a week 3. Several times a month 4. Once during these months
17) Nose bleed	☐1.Yes Today? Yes ___ No___ ☐ 2.No	1. Almost every day or every day 2. At least once a week 3. Several times a month 4. Once during these months
18) Stomach ache	☐1.Yes Today? Yes ___ No___ ☐ 2.No	1. Almost every day or every day 2. At least once a week 3. Several times a month 4. Once during these months
19) Ear ache	☐1.Yes Today? Yes ___ No___ ☐ 2.No	1. Almost every day or every day 2. At least once a week 3. Several times a month 4. Once during these months
20. **Extremely tired (much more than normal tiredness)**	☐1.Yes Today? Yes ___ No___ ☐ 2.No	1. Almost every day or every day 2. At least once a week 3. Several times a month 4. Once during these months
21. **Confusion**	☐1.Yes Today? Yes ___ No___ ☐ 2.No	1. Almost every day or every day 2. At least once a week 3. Several times a month 4. Once during these months

MATRIX FOR LIQUID CONSUMPTION

USUAL 24 HOURS LIQUID INTAKE								
Liquids consumed	**Sugar added**	**At home before work Litres or CC**	**Litres or CC brought from home to work and consumed**		**Litres or CC obtained or supplied at work**		**Litters or CC ingested after work**	**Observations**
			brought	**Consumed**	**Obtained/supplied**	**Consumed**		
Water								
Natural fruit drinks	None Little A lot							
Sodas								
Energy drinks								
Isotonic drink or bolis								
Coffee/ta	None Little A lot							
Milk	None Little A lot							
Other liquids: Soup _____								

Thank you!

Appendix C: Draft of a generic cohort protocol

Research paper cover sheet

RESEARCH PAPER COVER SHEET

PLEASE NOTE THAT A COVER SHEET MUST BE COMPLETED <u>FOR EACH</u> RESEARCH PAPER INCLUDED IN A THESIS.

SECTION A – Student Details

Student	Marvin Gonzalez-Quiroz
Principal Supervisor	Dorothea Nitsch
Thesis Title	Occupational kidney disease among young populations in northwest Nicaragua

<u>If the Research Paper has previously been published please complete Section B, if not please move to Section C</u>

<u>SECTION B – Paper already published</u>

Where was the work published?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	Choose an item.	Was the work subject to academic peer review?	Choose an item.

**If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.*

<u>SECTION C – Prepared for publication, but not to date published</u>

Where is the work intended to be published?	British Medical Journal (BMJ Open)
Please list the paper's authors in the intended authorship order:	Marvin Gonzalez-Quiroz, Dorothea Nitsch, Sophie Hamilton, Cristina O´Callaghan-Gordo, Ben Caplin, Neil Pearce, on behalf of the DEGREE Study Steering Committee

Stage of publication	**Not yet submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I developed this generic protocol based on the Nicaragua community-based cohort study with advice and supervision from Dorothea Nitsch, Ben Caplin and Neil Pearce. Sophie Hamilton and O´Callaghan-Gordo provided me support to develop the environmental section of baseline and follow-up questionnaire. The DEGREE study Steering Committee has reviewed the manuscript and gave me recommendations to improve it. After dealing with a political issue with the DEGREE study Steering Committee. I will send it to BMJ.

Student Signature: [redacted] **Date:** October 7/2018

Supervisor Signature: [redacted] **Date:** October 7/2018

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Rationale and community-based prospective cohort protocol for the Disadvantaged Populations at Risk of Decline in eGFR (CO-DEGREE)

Marvin Gonzalez-Quiroz, Dorothea Nitsch, Sophie Hamilton, Cristina O´Callaghan-Gordo, Ben Caplin, Neil Pearce, on behalf of the DEGREE Study Steering Committee

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on behalf of the DEGREE Study Steering Committee

**Equal contribution*

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This **original article** has been seen and approved by all authors listed above and is not under consideration for publication elsewhere.

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Abstract

Introduction

A recently recognised form of chronic kidney disease of unknown origin (CKDu) is afflicting communities in rural areas in several regions of the world. Prevalence studies are currently being conducted in a number of countries, using a standardised protocol, to estimate the distribution of glomerular filtration rate (GFR), and thus to identify communities where there is a high prevalence of reduced GFR. In many of these communities, cohort studies are now being planned to investigate the natural history of, and risk factors for, decline in kidney function over time. In this paper, we propose a standardised minimum protocol for such cohort studies in high risk communities.

Methods and analysis

This generic cohort protocol provides the information to establish a prospective community-based cohort study in low-income settings with high prevalence of CKDu. This involves a baseline survey that included key elements from the DEGREE survey methodology (e.g. using the previously published DEGREE methodology) of a population-representative sample, and subsequent follow-up visits in young adults (without known kidney disease (eGFR<60 mL/min/1.73 m^2) at baseline) over several years. Each visit involves a core questionnaire, and collection and storage of biological samples. Local capacity to measure serum creatinine (Scr) will be required so that immediate feedback on kidney function can be provided to participants. At the follow-up assessments repeat renal biomarkers should be measured in a central laboratory, using traceable to isotope dilution mass spectrometry (IDMS) traceable quality control to quantify

the main outcome of decline in kidney function over-time. A secondary aim of these studies is to investigate the possible risk factors for eGFR decline by quantifying exposures to potential causes of CKDu (by questionnaire or biosampling).

Ethics and dissemination

Ethical approval will be obtained by local researchers, and participants will provide informed consent before the study commences. All participants will receive feedback and advice on their laboratory results, and referral to a local health system where appropriate.

Trial registration number: Not applicable

Strengths and limitations of this study

- *We propose a prospective community-based generic cohort protocol for communities affected by CKDu in which the sampling frame consists of the entire at-risk population.*
- *Serial eGFR measurements in an apparently healthy population will allow the description of the natural history of disease and reduce problems associated with recall bias and reverse causation when assessing potential risk factors.*
- *Samples will be analysed in a single batch at local or international level at the end of the study to minimize time-dependent measurement errors.*
- *A biobank is expected to be created in each centre to store biological samples for future analyses*
- *The use of a standardised protocol will allow for regional and international comparisons*

Introduction

A recently recognised form of chronic kidney disease of unknown origin (CKDu) is afflicting rural communities in several regions of the world.[1-10] Several definitions for CKDu exist; the criteria typically include demonstration of renal damage using biomarkers in the absence of diabetes, severe hypertension or evidence of alternative renal diagnoses.[11-14] This disease has caused thousands of deaths and reduced the life expectancy among young adults in Mesoamerica, South Asia, and possibly in other tropical/subtropical regions of the world.[7, 15-19] The cause(s) of CKDu are not yet established, but proposed causes include recurrent dehydration from heat stress, pesticides, infections, and water contamination/heavy metals.[1, 20-22]

Although a broad range of cross-sectional studies of the prevalence of CKDu have been conducted in Mesoamerica, South Asia, and other regions of the world,[1-7, 9, 17] these have generally not use standardised methodology, and therefore do not allow for valid international comparisons. A recently published standardised protocol (the Disadvantaged Populations eGFR Epidemiology Study (DEGREE) protocol) for estimating the population distribution of glomerular filtration rate (eGFR), has addressed this concern, and its use will help to identify communities where there is a high prevalence of reduced eGFR. The DEGREE protocol makes it possible to undertake comparisons internationally, by mandating a population-representative sample and standardised collection of information on sociodemographic factors, occupational and environmental exposures, body composition and kidney function.[23] To date, studies using the DEGREE methodology have been

conducted in four countries (Peru, Sri Lanka, India, Malawi), with a number of further projects in preparation or in progress.[17]

A recent meta-analysis highlighted the lack of robust studies that have considered the natural history of CKDu.[24] We have therefore established a community-based cohort study to investigate the natural history of, and risk factors for, decline in kidney function over time.[25] Based on our experience[25, 26] we propose a generic cohort protocol to estimate the decline in kidney function over time and generate evidence of factors for loss of eGFR among at-risk populations. Our focus is on conducting such cohort studies in communities which are at high risk for CKDu that have been classified previously by cross-sectional study in high, medium or low prevalence based on a single or two GFR measurement. In general, such cohort studies would follow on from a cross-sectional survey using the DEGREE protocol, and hence we will use the term 'CO-DEGREE' (cohorts based on the DEGREE study) for such studies. In some situations, a DEGREE survey may form the 'baseline', with a subgroup of DEGREE survey participants then being selected for follow-up based on a single measurement of eGFR >60 mL/min/1.73 m^2 and without clinical diagnosis or history of hypertension, diabetes mellitus or obesity. However, the standardised protocol we propose here can also be used as a 'stand-alone' study design, without requiring that a DEGREE survey is conducted first.

We are already conducting such a cohort study in Nicaragua,[25, 26] and have had many challenges to address, including: (i) community engagement, awareness of conditions, political unrest and ethics; (ii) follow-up over time

(frequency and minimising loss to follow-up); (iii) fieldwork and laboratory standards to ensure decline is detected; and (iv) regular feedback information on study progress. We will draw on our experience in Nicaragua in presenting both the generic CO-DEGREE protocol, as well as observations on the practical issues involved in conducting such studies in a particular community.

Objectives

Studies using this generic cohort protocol, and contributing to the wider DEGREE collaboration, will aim to:

1. investigate the natural history of kidney function decline over time among populations at risk of CKDu
2. identify risk factors for kidney function decline in these populations, to better inform further in-depth aetiological studies and direct future preventative strategies
3. compare the natural history, and risk factors for kidney function decline, in different communities and regions at risk of CKDu
4. establish a framework for international collaboration and promote a network for future work on the causality of CKDu

Rationale for a community cohort study of decline in eGFR

A representative sample of those at-risk

Community-based cohort studies have several advantages:[27] Firstly this type of study allows the recruitment of a representative sample of the at-risk population, e.g. it will include workers from a variety of occupations (including not economically active) at the community level. Assuming that the study

sample is randomly selected from the entire at-risk population, and there are no substantial problems with non-response, these studies are unlikely to be affected by significant selection bias. This is in contrast to occupationally-based studies where the healthy worker effect can be a major problem, particularly if workers are screened for kidney disease prior to starting employment because it unlikely to observe the effect in this group due to a small number/null of cases during the study period.[28] One general disadvantage of community-based studies is that this approach typically requires large sample sizes and long-term follow-up if disease is not highly prevalent. However, the focus of CO-DEGREE is on conducting studies in communities with a high prevalence of CKDu (see below).[26, 27]

Handling reverse causation and recall bias

Reverse causality in cross-sectional studies arises when participants at risk modify their lifestyle, behavioural or working exposures due to the outcome of interest (e.g. renal dysfunction). For example, someone with impaired kidney function may leave employment (or may be screened and then denied continued employment), in which case their exposures (e.g. to heat stress or pesticides) will change. This problem can be avoided, or minimised, in a cohort study by excluding people with pre-existing disease, and then following the remaining 'healthy' participants over time. Similarly, cross-sectional studies may be prone to recall bias regarding previous exposures.

Measuring kidney function

Quantification of kidney function is most easily undertaken by determining the serum creatinine (Scr) concentration, which is relatively easy and cheap to measure, and then calculating the eGFR. A case of CKDu is typically defined by an eGFR <60 mL/min/1.73 m^2 (sustained for at least 3 months where chronicity is confirmed) in the absence of known causes of kidney disease. However, this dichotomous definition of CKDu has weaknesses in studies exploring the causation of CKDu, as it is well established that substantial damage may have already occurred at the histological level before serum biomarkers of renal dysfunction become definitively abnormal (and other markers such as proteinuria are often absent in this disease). Furthermore, Scr levels are modified by multiple non-renal factors such as: high animal protein-intake, strenuous exercise, changes in plasma volume, body mass index (BMI), sex, age, ethnicity, and some drugs;[29] thus, cross-sectional studies examining associations with reduced eGFR based on a single Scr measurement may be prone to a significant degree of misclassification. In addition, the CKD-EPI or MDRD equation used to calculate eGFR from serum creatinine,[29] have not been validated in many populations reported to be suffering CKDu,[30] potentially further increasing misclassification bias in cross-sectional studies.

Alternative approaches based on serial eGFR measurements in the same person over time render between-person variation less problematic. If estimated across a period of time using multiple measures, this will also reduce the influence of the within-person factors that are not directly related to kidney damage. The successful measurements of eGFR over time can admit a dropout

less than 30% of the original sample size. In summary, an approach utilising serial eGFR measures substantially increases the potential utility to identify risk/causal factors for CKDu as well as allowing the description of the natural history of disease.

Core protocol

Study design

This is a prospective cohort study protocol for studying decline in kidney function over time in populations with high reported prevalence of CKDu, primarily in low- and middle- income countries (LMICs). We consider the following study design issues: (i) population sampling strategy, and follow-up interval (ii) questionnaire development and delivery, (iii) clinical measurements and biosampling, and (iv) data management and reporting.[26] (See figure 1) In addition, we discuss: (a) sample size and follow-up duration; and (b) ethical considerations.

Population, sampling strategy and follow-up interval

In Mesoamerica, CKDu typically affects young men on the Pacific Coast. This population is dying often younger or in their 40s from end stage renal disease.[15, 31] The disease appears to occur at a later age in South Asia, with few cases occurring in men in their 20s.[7, 32] Nevertheless, one might expect preliminary changes in GFR to occur early in adulthood. In general, the study population should include participants who are old enough to experience an identifiable decline in kidney function, but not older age-groups (> 50 year-old) where the prevalence of CKD is already high in almost all populations globally

(e.g. greater than 10%). Thus, inclusion criteria should be tailored to the local disease profile, but the default approach should be to recruit participants aged 18-40 years-old (though 18-30 might be more appropriate in Central America, and 18-50 may be more appropriate in areas such as Sri Lanka where age of onset appears older). A community-census should be conducted to identify all potential participants in the appropriate age range and either the entire population recruited, or a random sample selected. In either case, response rates by age and sex, should be reported.

The focus is on studying participants who are 'at risk' of CKDu, i.e. they do not already have CKD or factors which would exclude a CKDu diagnosis (e.g. diabetes mellitus, hypertension) and pregnancy. Thus, the sample size estimates (see below) are based on following a cohort in which those where a pre-existing diagnosis of CKD, diabetes or hypertension have been excluded.[26] For practical, ethical or scientific reasons, one may wish to study an entire community (including those with pre-existing clinical diagnosis of CKD, diabetes mellitus (fasting serum glucose: >105 mg/dL) and hypertension (blood pressure >140/90 mmHg)), but in that case it is important to ensure that there are sufficient 'disease free' participants to meet the sample size requirements. Although the disease is generally more common in men, women with CKDu are of strong scientific interest in that they may suggest alternative risk factors, or help to rule out some that have been previously proposed. Hence recruitment should in general involve equal numbers of males and females.

The baseline study visit will require the administration of the core-questionnaire, clinical measurements and biological samples. Subsequent to the baseline visit, follow-up visits should be conducted at least annually for a minimum follow-up of two-years to evaluate the study outcome and keep close contact with the participants and update their contact information. This will help minimize the loss to follow-up at each study point. Substantial seasonal variation in eGFR has been reported in a number of settings (both CKDu related and unrelated). Therefore, the conduct of additional study visits at a 6-monthly interval (at beginning and end of summer season) might be useful in explaining within-person eGFR variation as well as providing important information for the wider population on the significance of kidney function testing at different time point in the year (perhaps for a subset of participants or a proportion of the follow-up period).

Questionnaires

The purpose of the baseline core-questionnaire is to obtain a minimum dataset to explore associations with decline kidney function and make comparisons within and between persons. The baseline core-questionnaire (supplementary file 1) is based on the questionnaire used in our study in Nicaragua, and has been further developed by combining questionnaires that have been used in DEGREE-related studies in a number of settings. The baseline core-questionnaire represents a minimum data set, and local research teams may decide to add data items of specific interest to the core dataset. The as well have the responsibility to translate, validate, and to make any local contextual

changes. Additional modules are under development by the DEGREE study collaboration (e.g. an environmental exposure questionnaire).

Researchers will return annually for in-person follow-up visits. All participants have to respond a follow-up questionnaire (supplementary file 2) and update their contact information.

Clinical measurements

Blood pressure should be measured after 5 minutes rest in the sitting position using an automated sphygmomanometer and the average of three readings recorded. Subjects height and weight (in centimetres and kilograms) should be measured (without shoes) using a stadiometer and digital calibrated scales.

Biosamples

Blood and urine samples will be collected at each study visit and stored in the field into dry ice or liquid nitrogen after collection or in a worse scenario store in coolers with icebox (4ºC) no more than 3 hours.

Dipstick urinalysis should be performed by using electronic readers (urine chemistry analyzer) where possible to use it or if not possible at least 10% of urine dipstick analysed by a lab technician should be re-analysed by other reader. The minimum of parameters that should be reported are: specific urinary gravity, pH, protein, blood, leucocytes, and glucose.

Samples for serum analysis should be centrifuged at 3500 rpm for 10 minutes within 3 hours of collection, and subsequently separated into four aliquots of 1-2

mL each one and stored at -80ºC (or -20ºC if not possible). One aliquot should be used for contemporary local serum creatinine measurements e.g. by using the modified Jaffe assay (traceable to isotope dilution mass spectrometry [IDMS] reference standards if possible). At baseline and during each study visit a cross-checking of local lab quality control is highly recommended to ensure that Scr determinations are comparable. Also, lab results will guide clinical care for participants during the follow-up period.

A further aliquot should be stored for batch measurement of serum creatinine at the end of follow-up using a method traceable to an IDMS reference material. The CO-DEGREE group suggest the storage of at least three 1-2mL aliquots of serum and a similar amount of urine in addition to those described above. Additional samples and analyses should be pursued depending on the priorities of the local research team. All samples for future analysis should be stored at -20ºC or ideally at -80ºC in a local or international biobank. This biobank needs to secure an uninterruptible power supply to protect the samples because loss of electricity for even a few days will ruin the samples.

Investigators should assess and obtain consent from participants for future use of samples for further analyses both locally and internationally (e.g. through the DEGREE collaboration) as well as ensure that storage capacity is available.

Data management and reporting

Questionnaires and samples will be labelled using a unique bar-code to keep the participants confidentiality. Electronic data capture systems such as Open

Data Kit Software[33] may be the most resource efficient method to capture questionnaire data but where hard-copies are used double data-entry should be undertaken to minimise the transcription errors.

The CO-DEGREE protocols are openly available to interested research teams. Each centre will be owner of their data and expected to publish the results of their study independently. However, where a study is registered as part of the DEGREE collaboration the group will request a digital copy of anonymized individual-level data, with a guideline for basic contextual information of local setting and a description of the population characteristics should be elaborated, for the DEGREE data centre to conduct international comparisons.

Sample size and follow-up duration

The overall scale of the cohort will be largely dependent on the proportion of the 'healthy' population which is expected to experience a 'substantial' decline in eGFR over time in the community as a result of CKDu. As discussed above, demonstrating that reduced renal function without diabetes, hypertension or known kidney diseases is prevalent on a cross-sectional basis is a necessary first step before pursuing this work. If for example this study protocol was to be conducted in a general population sample in Europe or the USA with similar exclusion criteria, then CKDu would be virtually non-existent, and there would also be very little or no decline of kidney function. In contrast, in our Nicaragua study of apparently healthy adults aged 18-30 years,[25, 26] there was a clearly distinct subgroup which experienced a marked decline in kidney function over time, whereas the eGFR in the other study participants was relatively stable.

Given this distribution of eGFR trajectories in the population we would expect the analysis to be conducted using a prospective case-control approach.

Therefore, the sample size requirements to detect an association with an exposure at any given power will be determined by the following factors:

1. Proportion of the population that experience 'substantial' decline

    In turn the power to detect 'substantial' decline will depend on:

    a) The rate of eGFR decline in those affected

    b) The duration of follow-up

    c) The number of eGFR measures

2. Proportion of general population exposed to any exposure of interest
3. Effect size of any exposure
4. The study retention rate

Taking a simplistic approach, the duration of the study should be designed so that those affected have sustained a clinically important loss of kidney function, e.g. in our Nicaragua study this was 30% over two-years.[34] Therefore, if CKDu in the study population is predicted, from a normal baseline, to lead to a loss of eGFR of a magnitude of 5% each year (~7mL/min/1.73 m^2/year) the study duration should be 4 years. If alternatively, loss is predicted to 10% each year study duration could be as short as 2 years. Additional eGFR measures, over and above the suggested annual frequency will reduce error associated with determining trajectory (and might be performed for the reasons discussed above) but either way a minimum follow-up of 2 years is recommended.

After basing the study duration on the expected rate of eGFR decline among those affected, the sample size can then be calculated on the basis of the expected frequency of 'substantial' decline amongst the population and the effect size of any proposed exposure that it is desirable to detect. A number of scenarios are outlined in Table 2. A further (10-20%, depending on local circumstances) increase in target recruitment is advised to allow for loss to follow-up.

Finally, these initial sample size estimates will need further adjustment for exclusions following the detection of previously unknown CKD (based on eGFR/albuminuria tests), or newly detected diabetes or hypertension at baseline (unless these data are available from a previously conducted cross-sectional study). It is worth considering whether people who may have CKDu (eGFR <60 mL/min/1.73 m^2) will be aware of it, as this will affect how many people need to be tested prior to recruitment into the cohort. If there is screening for kidney problems (as for example in Nicaraguan Sugarcane mills), then potential cohort participants may be aware of their kidney function status and can be excluded from the cohort prior to the baseline visit. This occurred for example for 5% of the target population in the community studied in Nicaragua. Nevertheless, there was an additional 10% who had undiagnosed impaired kidney function at baseline assessment based on their laboratory records, highlighting the importance of choosing an age-group where CKDu is not already highly prevalent so as to easily satisfy a key inclusion criterion (absence of CKD at baseline).

Ethics/regulatory issues and dissemination

Local research teams will ensure these studies are conducted in accordance with the Declaration of Helsinki Principles and be responsible for assuring that the work is approved by the local institutional review board (IRBs). Written informed consent will be obtained from all participants before taking part in the study. Information should be transparent in terms of using the data and stored biosamples stored for future research. A key aspect of the ethical review of any protocol is ensuring the adequate provision of feedback and advice to participants when abnormal results become available. Furthermore, mechanisms will need to be developed in partnership with local health providers/healthcare systems to handle participants needing referral for medical care. Findings from these studies should be disseminated widely by publication in peer-reviewed journals and presentations/representations to relevant local stake holders.

Experience with the CO-DEGREE protocol in Nicaragua

The protocol presented here is, by necessity, very generic. The approaches and challenges of implementing the protocol will vary widely in different communities and regions of the world. However, since we have already implemented this protocol in a study in Nicaragua,[25, 26] we will make some observations on the practicalities, and challenges, or implementing the protocol in this context.

The Nicaragua study involved community-based follow-up in Leon and Chinandega departments. [26] A number of strategies were used to maximise response and retention rates. As the workday starts very early in the morning

and finishes late in the afternoon attempts were made to conduct data collection during economically less active (e.g. each side of the main harvest) periods of the year, so as to still capture participants who were employed at the time. Additionally, participants receive their kidney test results within a fortnight of the study visits, and receive reimbursement of expenses and any lost income they have incurred to attend the study visit. Although study visits have been timetabled to occur outside of the harvest season, employees still express the concern that their employment opportunities might be affected by taking part in the study. In an attempt to mitigate against these types of consequences, the study team have corresponded with local employers explaining the content and extent of this study in order to reduce any concerns about workers' participation. In addition, the study team takes particular precautions to maintain participant's confidentiality during the study and beyond.

Conducting a follow up study in a rural area remains a major challenge. Alongside the logistical challenges of reaching geographically isolated neighbourhoods along poor quality roads, a significant obstacle has been internal and external migration due to lack of employment source or social unrest. Rural communities have a tradition of working with seasonal crops and sugarcane workers often leave their communities at the end of each harvest season, to go abroad or to other regions within the country in search of temporary employment. At the end of each harvest, up to 30% of the study population left their communities in search of alternative employment during the non-harvest period in our study. Despite these problems our team achieved

92% for follow-up at two years.[25, 26] but this has required the investment of significant time and resources.

Finally, continuing community engagement and the maintenance of good relationships between researchers, community leaders, participants and communication with local health care system have been key. E.g., It is very important to develop a reference flowchart and communication with local health posts/primary hospital or hospital for persons with health problems detected during the study.

Discussion

The CO-DEGREE protocol was developed in response to the highly prevalent form of CKD of unknown cause that is affecting Mesoamerica and other countries around the globe. To date, the existing epidemiological studies of CKDu have provided an incomplete understanding of the natural history of and risk factors for disease. This CO-DEGREE protocol aims to provide a framework to address this.

This CO-DEGREE protocol is designed to capture the entire at-risk population by aiming to recruit men and women, and those that work across a variety of different occupations. The main outcome measure of within-person loss of eGFR over time means it is not only possible to describe the natural history of disease but also to capture the earliest disease stages of disease, making associations with possible exposures less prone to reverse causation and recall bias.

We do not underestimate the challenges posed by the lack of standardized exposure questionnaires in this area. This protocol uses a questionnaire that combines questions from a number of sites (Nicaragua, and various DEGREE sites) aimed at capturing a variety of exposures, including sociodemographic data, occupational and environmental exposures, lifestyle factors, heat-related symptoms, etc. both in agricultural and non-agricultural settings. However, in the absence of globally generalisable instruments, short or long-term environmental measurements and/or novel biomarkers that capture exposure to heat, agrichemicals, and/or infection in either the community or workplace are likely to be valuable additions to this type of study but are beyond the scope of this basic protocol.

Finally, it should be emphasized that this protocol is not suitable for studying progression of CKD in general, due to the specific constraints introduced by excluding those with hypertension, diabetes and CKD (i.e. those with proteinuria and/or with reduced eGFR) at baseline. In settings where there is no a high prevalence of CKDu, a cohort comprised of people without traditional risk factors for CKD or with CKD would be unlikely to identify any detectable kidney function loss over time in the target population. For studies outside the CKDu arena, investigators are advised to use alternative methodologies using established protocols, for example, the CRIC study.[35]

In conclusion, we have designed a CO-DEGREE protocol that can be used in the different settings around the globe to investigate the natural history of CKDu and associated risk factors for decline in kidney function. These studies should

provide important information on the rate of decline of kidney function across different affected areas as well as key insight into the cause(s) of disease.

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References

1. Wegman D, Crowe J, Hogstedt C, Jakobsson K, Wesseling C, editors. Mesoamerican nephropathy: report from the second international research workshop on MeN. Heredia, C.R: SALTRA/IRET-UNA; 2016. Report No.: ISBN 978-9968-924-33-7.

2. Torres C, Aragon A, Gonzalez M, Lopez I, Jakobsson K, Elinder CG, et al. Decreased kidney function of unknown cause in Nicaragua: a community-based survey. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2010;55(3):485-96.

3. O'Donnell JK, Tobey M, Weiner DE, Stevens LA, Johnson S, Stringham P, et al. Prevalence of and risk factors for chronic kidney disease in rural Nicaragua. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2011;26(9):2798-805.

4. Orantes CM, Herrera R, Almaguer M, Brizuela EG, Hernandez CE, Bayarre H, et al. Chronic kidney disease and associated risk factors in the Bajo Lempa region of El Salvador: Nefrolempa study, 2009. MEDICC review. 2011;13(4):14-22.

5. Orantes CM, Herrera R, Almaguer M, Brizuela EG, Nunez L, Alvarado NP, et al. Epidemiology of chronic kidney disease in adults of Salvadoran agricultural communities. MEDICC review. 2014;16(2):23-30.

6. Jayasekara JM, Dissanayake DM, Adhikari SB, Bandara P. Geographical distribution of chronic kidney disease of unknown origin in North Central Region of Sri Lanka. The Ceylon medical journal. 2013;58(1):6-10.

7. Jayatilake N, Mendis S, Maheepala P, Mehta FR, Team CKNRP. Chronic kidney disease of uncertain aetiology: prevalence and causative factors in a developing country. BMC Nephrol. 2013;14:180.

8. Ganguli A. Uddanam Nephropathy/Regional Nephropathy in India: Preliminary Findings and a Plea for Further Research. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2016;68(3):344-8.

9. Jayasumana C, Orantes C, Herrera R, Almaguer M, Lopez L, Silva LC, et al. Chronic interstitial nephritis in agricultural communities: a worldwide epidemic with social, occupational and environmental determinants. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2017;32(2):234-41.

10. Peraza S, Wesseling C, Aragon A, Leiva R, Garcia-Trabanino RA, Torres C, et al. Decreased kidney function among agricultural workers in El Salvador. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2012;59(4):531-40.

11. García-Trabanino R, Cerdas M, Madero M, Jakobsson K, Barnoya J, Crowe J, et al. Nefropatía mesoamericana: revisión breve basada en el segundo taller del Consorcio para el estudio de la Epidemia de Nefropatía en Centroamérica y México (CENCAM). Nefrología Latinoamericana. 2017;14(1):39-45.

12. Lozier M, Turcios-Ruiz RM, Noonan G, Ordunez P. Chronic kidney disease of nontraditional etiology in Central America: a provisional epidemiologic case definition for surveillance and epidemiologic studies. Revista panamericana de salud publica = Pan American journal of public health. 2016;40(5):294-300.

13. Rajapakse S, Shivanthan MC, Selvarajah M. Chronic kidney disease of unknown etiology in Sri Lanka. International journal of occupational and environmental health. 2016;22(3):259-64.

14. WHO. Workshop report: Designing a step-wise approach to estimate the burden and to understand the etiology of CKDu in Sri Lanka. Sri Lanka: WHO; 2016, 24-25th October.

15. Ordunez P, Nieto FJ, Martinez R, Soliz P, Giraldo GP, Mott SA, et al. Chronic kidney disease mortality trends in selected Central America countries, 1997-2013: clues to an epidemic of chronic interstitial nephritis of agricultural communities. Journal of epidemiology and community health. 2018;72(4):280-6.

16. Ordunez P, Saenz C, Martinez R, Chapman E, Reveiz L, Becerra F. The epidemic of chronic kidney disease in Central America. The Lancet Global health. 2014;2(8):e440-1.

17. Ekiti ME, Zambo JB, Assah FK, Agbor VN, Kekay K, Ashuntantang G. Chronic kidney disease in sugarcane workers in Cameroon: a cross-sectional study. BMC Nephrol. 2018;19(1):10.

18. Ministry of Health Nutrition and Indigenous Medicine - Medical Statistics Unit. Annual health bulletin of Sri Lanka 2015. Sri Lanka: Ministry of Health, Nutrition and Indigenous Medicine; 2017 [cited 2017 June 20]. Available from: http://www.health.gov.lk/moh_final/english/public/elfinder/files/publications/AHB/2017/AHB%202015.pdf.

19. Nanayakkara S KT, Rajapurkar MM, John GT, Kirpalani AL. . What do we know about chronic kidney disease in India: first report of the Indian CKD registry. . BMC Nephrol 2012;13(10).

20. Correa-Rotter R, Wesseling C, Johnson RJ. CKD of unknown origin in Central America: the case for a Mesoamerican nephropathy. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2014;63(3):506-20.

21. Wesseling C, Crowe J, Hogstedt C, Jakobsson K, Lucas R, Wegman DH. The epidemic of chronic kidney disease of unknown etiology in Mesoamerica: a call for interdisciplinary research and action. American journal of public health. 2013;103(11):1927-30.

22. Wesseling C, Crowe J, Hogstedt C, Jakobsson K, Lucas R, Wegman D, et al. First international research workshop on mesoamerican nephropathy (MeN). Heredia, C.R.: SALTRA/IRET-UNA; 2013. Report No.: ISBN 978-9968-924-06-1.

23. Caplin B, Jakobsson K, Glaser J, Nitsch D, Jha V, Singh A, et al. International Collaboration for the Epidemiology of eGFR in Low and Middle Income Populations - Rationale and core protocol for the Disadvantaged Populations eGFR Epidemiology Study (DEGREE). BMC Nephrol. 2017;18(1):1.

24. Gonzalez-Quiroz M, Pearce N, Caplin B, Nitsch D. What do epidemiological studies tell us about chronic kidney disease of undetermined cause in Mesoamerica?: A systematic review and meta-analysis. Clinical Kidney Journal. 2017:1-11.

25. Gonzalez-Quiroz M, Smpokou ET, Silverwood RJ, Camacho A, Faber D, Garcia BR, et al. Decline in Kidney Function among Apparently Healthy Young Adults at Risk of Mesoamerican Nephropathy. J Am Soc Nephrol. 2018;29:2200–12.

26. Gonzalez-Quiroz M, Camacho A, Faber D, Aragon A, Wesseling C, Glaser J, et al. Rationale, description and baseline findings of a community-based prospective cohort study of kidney function amongst the young rural population of Northwest Nicaragua. BMC Nephrol. 2017;18(1):16.

27. Caplin Ben, González-Quiroz Marvin, Pearce Neil. Gaining insights into the evolution of CKDnt from community-based follow up studies. In: SALTRA, editor. Second International Workshop on Mesoamerican Nephropathy; San José. Costa Rica: SALTRA; 2015.

28. Checkoway Harvey, Pearce Neil, Kriebel David. Research methods in occupational epidemiology. Second ed. Press OU, editor. New York: Oxford University Press, Inc.; 2004. 372 p.

29. Padala S, Tighiouart H, Inker LA, Contreras G, Beck GJ, Lewis J, et al. Accuracy of a GFR estimating equation over time in people with a wide range of kidney function. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2012;60(2):217-24.

30. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. The New England journal of medicine. 2012;367(1):20-9.

31. Garcia-Trabanino R, Trujillo Z, Colorado AV, Magana Mercado S, Henriquez CA, En nombre de la Asociacion de Nefrologia e Hipertension Arterial de El S. Prevalence of patients receiving renal replacement therapy in El Salvador in 2014. Nefrologia : publicacion oficial de la Sociedad Espanola Nefrologia. 2016;36(6):631-6.

32. Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, et al. Chronic kidney disease: global dimension and perspectives. Lancet. 2013;382(9888):260-72.

33. Open Data KIT. Longitudinal Clinic Study App: GitHub, Inc; 2018 [cited 2018 July 28]. Available from: https://opendatakit.org/use/2_0_tools/odk-application-designer-2-0-rev126/.

34. Coresh J, Turin TC, Matsushita K, Sang Y, Ballew SH, Appel LJ, et al. Decline in estimated glomerular filtration rate and subsequent risk of end-stage renal disease and mortality. Jama. 2014;311(24):2518-31.

35. Feldman HI, Appel LJ, Chertow GM, Cifelli D, Cizman B, Daugirdas J, et al. The Chronic Renal Insufficiency Cohort (CRIC) Study: Design and Methods. J Am Soc Nephrol. 2003;14(7 Suppl 2):S148-53.

Figure 1: Flow chart and study procedures of CO-DEGREE protocol.

Table 1. Details and procedures of the baseline study visit and subsequence follow-up

Items	***Baseline visit (0 month)***	***Follow-up period (variable)***				
		12 months	*24 months*	*36 months*	*48 months*	*At completion*
Community census	X	-	-	-	-	-
Participants enrolment	X	-	-	-	-	-
Informed consent	X					
Update personnel contact information	X	X	X	X	X	
Anthropometric measurements	X	X	X	X	X	
Biological samples	X	X	X	X	X	
Baseline core-questionnaire	X	-	-	-	-	
Follow-up questionnaire		X	X	X	X	
Local serum creatinine measurement	X	X	X	X	X	
Results feedback	X	X	X	X	X	
Biobank	X	X	X	X	X	
Batch testing of serum creatinine						X

Table 2: Sample Size Calculations

Parameters	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6	Scenario 7	Scenario 8
Population frequency of eGFR decline	0.04	0.06	0.08	0.10	0.04	0.06	0.08	0.10
Proportion population exposed	0.5							
Odds ratio associated with exposure	2				3			
P (oucome|unexposed)	0.027	0.04	0.053	0.066	0.02	0.03	0.04	0.05
P (oucome|exposed)	0.054	0.08	0.106	0.132	0.06	0.09	0.12	0.15
Group size	993	686	405	436	463	317	243	200
Sample size	**1986**	**1372**	**810**	**872**	**926**	**634**	**486**	**400**

Abbreviations, eGFR, estimated glomerular filtration rate; P: probability. Assumes 1-β=0.80; =0.05; Calculations based on equal proportion of the population exposed/unexposed for simplicity. No adjustments made for loss to follow-up or multiple testing.

Appendix D: A list of associated papers have not been included in the thesis

AJKD
Original Investigation

Renal Morphology, Clinical Findings, and Progression Rate in Mesoamerican Nephropathy

Julia Wijkström, MD,[1] Marvin González-Quiroz, MD, MSc,[2,3] Mario Hernandez, MD,[4] Zulma Trujillo, MD,[5] Kjell Hultenby, PhD,[6] Anneli Ring, BS,[7] Magnus Söderberg, MD, PhD,[7,8] Aurora Aragón, MD, PhD,[2] Carl-Gustaf Elinder, MD, PhD,[1] and Annika Wernerson, MD, PhD[1,7]

Background: Mesoamerican nephropathy (MeN) is a chronic kidney disease affecting rural inhabitants in Central America. We have previously described the renal morphology in 8 patients from El Salvador. To confirm the renal pathology, we have studied kidney biopsies from patients with MeN in Nicaragua. Follow-up urine and blood samples from both biopsy studies were collected to investigate the natural history.

Study Design: Case series.

Settings & Participants: In the kidney biopsy study, 19 male sugarcane workers in Nicaragua with suspected MeN were investigated with questionnaires, kidney biopsies, and blood and urine analysis. Inclusion criteria were age 20 to 65 years and plasma creatinine level of 1.13 to 2.49 mg/dL or estimated glomerular filtration rate (eGFR) of 30 to 80 mL/min/1.73 m^2. Exclusion criteria were proteinuria with protein excretion > 3 g/24 h, uncontrolled hypertension, diabetes mellitus, or other known kidney disease. In the follow up-study, blood and urine from the kidney biopsy study in Nicaragua (n = 18) and our previous biopsy study of MeN cases in El Salvador (n = 7) were collected 1 to 1.5 and 2 to 2.5 years after biopsy, respectively.

Outcomes: Renal morphology, clinical, and biochemical characteristics, change in eGFR per year.

Measurements: eGFR was calculated using the CKD-EPI creatinine ($eGFR_{cr}$), cystatin C ($eGFR_{cys}$), and creatinine-cystatin C ($eGFR_{cr\text{-}cys}$) equations.

Results: In the kidney biopsy study, participants had a mean $eGFR_{cr}$ of 57 (range, 33-96) mL/min/1.73 m^2. 47% had low plasma sodium and 21% had low plasma potassium levels. 16 kidney biopsies were representative and showed glomerulosclerosis (mean, 38%), glomerular hypertrophy, and signs of chronic glomerular ischemia. Mild to moderate tubulointerstitial damage and mostly mild vascular changes were seen. In the follow up-study, median duration of follow-up was 13 (range, 13-27) months. Mean change in $eGFR_{cr}$ was -4.4 ± 8.4 (range, -27.7 to 10.2) mL/min/1.73 m^2 per year. Most patients had stopped working with sugarcane cultivation.

Limitations: 3 biopsy specimens had 4 or fewer glomeruli.

Conclusions: This study confirms the renal morphology of MeN: chronic glomerular and tubulointerstitial damage with glomerulosclerosis and chronic glomerular ischemia. Follow-up data show that eGFRs, on average, deteriorated.

Am J Kidney Dis. 69(5):626-636.

INDEX WORDS: Mesoamerican nephropathy (MeN); chronic kidney disease (CKD); renal pathology; renal morphology; kidney biopsy; Central America; heat stress; dehydration; sugarcane; environmental exposure; CKD of unknown etiology (CKDu); endemic nephropathy; disease progression; Nicaragua; El Salvador.

Mesoamerican nephropathy (MeN), an endemic form of chronic kidney disease (CKD), affects rural inhabitants in Central America.[1-3] Cross-sectional studies in Nicaragua and El Salvador have shown a high prevalence of CKD in villages near the Pacific coast, where agricultural work and other types of manual labor are the main source of employment.[4,5] Men working in sugarcane fields are often

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affected and the disease may progress to chronic kidney failure, a devastating diagnosis in most Central American countries, where the resources for renal replacement therapy are limited. The cause and pathogenesis of MeN are still not fully known, but the extreme occupational conditions of the sugarcane workers have made repeated dehydration the leading hypothesis.[6,7]

Patients with MeN present with elevated creatinine levels, no hypertension, and urine albumin levels are normal or of non-nephrotic range. In 2012, we performed the first study on kidney biopsies and clinical presentations of 8 agricultural workers in El Salvador with MeN.[8] We found a distinctive renal morphology with widespread glomerulosclerosis and signs of chronic glomerular ischemia, but only mild to moderate tubulointerstitial changes. To confirm these findings in a larger cohort and in another area, we have designed the present study in Nicaragua. To examine the progression rate and possible prognostic factors in MeN, we have collected follow-up blood and urine samples from patients in the present biopsy study and also from our previous biopsy study in El Salvador.[8]

METHODS

Nicaragua Kidney Biopsy Study

Men with CKD of unknown cause and a history of sugarcane work who had been to a medical visit at the Research Center of Health, Work and Environment, National Autonomous University of Nicaragua at León during a 12-month period were selected as possible participants. Inclusion criteria were age 20 to 65 years and plasma creatinine level of 100 to 220 μmol/L (1.13-2.49 mg/dL) or estimated glomerular filtration rate (eGFR) of 30 to 80 mL/min/1.73 m^2. Exclusion criteria were proteinuria with protein excretion > 3 g/24 h, uncontrolled hypertension (blood pressure > 140/90 mm Hg or >1 antihypertensive drug), diabetes mellitus (blood glucose > 126 mg/dL), or other known kidney disease.

The study was performed in May 2014 (ie, after sugarcane harvest season). Kidney biopsies and blood, urine, and clinical data were collected. Ultrasound examinations of kidneys were carried out before the biopsy. Participants answered questionnaires (both open- and closed-ended questions) about employment (years in different occupations); exposure to chemicals or pesticides (yes/no); fluid intake (volume and type of fluid consumed); current medicine use and estimation of weekly intake of nonsteroidal anti-inflammatory drugs (NSAIDs), acetaminophen, and other analgesics during different periods in their lifetime; smoking habits (past or current smoking); and if they had ever fainted at work (yes/no).

Blood and Urine Samples

Within 1 week prior to the kidney biopsy procedure, blood and urine samples were collected and analyzed at the laboratory of the faculty of medicine at National Autonomous University of Nicaragua at León for hepatitis B and C virus, hematocrit, white blood cell count, international normalized ratio, activated partial thromboplastin time, and urine dipstick, sediment, culture, and 24-hour protein.

At the morning of the biopsy, new blood and urine samples were collected and stored at −20°C for 1 to 4 days, then shipped on dry ice to Karolinska Institutet and stored at −70°C. At Karolinska University Hospital Laboratory (KUHL), plasma was analyzed for creatinine, cystatin C, potassium, sodium, uric acid, calcium, albumin, alanine aminotransferase, antineutrophil cytoplasmic antibody and antinuclear antibody screening, anti−glomerular basement membrane (GBM) antibodies, and complement. Urine was analyzed for albumin, creatinine, uric acid, sodium, potassium, *N*-acetyl-β-D-glucosaminidase (NAG), and α_1-microglobulin. All analyses were performed according to standard protocols. The creatinine and cystatin C assays used were isotope-dilution mass spectrometry (IDMS) traceable. Urine arsenic, cadmium, mercury, and lead were analyzed with inductively coupled plasma mass spectrometry according to US Environmental Protection Agency methods 200.8[9] at ALS Scandinavia AB Laboratory, Luleå, Sweden. When calculating mean values, urinary values below the lower limits of detection were imputed as one-half the lower limit of detection value; albumin < 3.0 mg/L was converted to 1.5 mg/L; NAG < 0.5 nkat/L, to 0.25 nkat/L; α_1-microglobulin < 6 mg/L, to 3 mg/L; cadmium < 0.05 μg/L, to 0.025 μg/L; mercury < 0.2 μg/L, to 0.1 μg/L, and lead < 0.5 μg/L, to 0.25 μg/L. eGFR was calculated using the CKD-EPI (CKD Epidemiology Collaboration) equations for creatinine ($eGFR_{cr}$), cystatin C ($eGFR_{cys}$), and creatinine−cystatin C ($eGFR_{cr\text{-}cys}$).[10,11] Reference values are presented in the tables and were according to KUHL, except for plasma potassium and uric acid (from the Nicaragua laboratory). For urine heavy metal reference range, see references 12 and 13.

Kidney Biopsies

Percutaneous ultrasound-guided kidney biopsies were performed at Hospital La Fraternidad, filial San José, using a spring-loaded needle (16 Ga Biocore II MG/DANA 2.2 MG; Histo). After the biopsy, patients stayed for 24-hour observation. Biopsy specimens were divided and prepared according to standard protocols for light microscopy, immunofluorescence, and electron microscopy (for details see Item S1, available as online supplementary material). De Galantha staining was performed on paraffin-embedded tissue to detect urate crystals.[14]

Two senior consultants in renal pathology (A.W. and M.S.) did individual evaluations of the renal morphology followed by a discussion to reach consensus.

Biopsy specimens with 10 or more glomeruli were rated as representative; 7 to 9 glomeruli, as marginally representative; and fewer than 7 glomeruli, as not representative. Grading of tubular atrophy, interstitial inflammation, and interstitial fibrosis in the cortical area were defined as follows: mild, affecting <25% of the area; moderate, affecting 26% to 50%; and severe, affecting >50%.[15] Glomerular hypertrophy and vascular pathology were graded semiquantitatively as no/normal, mild, moderate, or severe.

Podocyte foot-process effacement was semiquantified by calculating the number of slits per micron of GBM in 5 random capillaries per glomerulus. Effacement was defined as widespread, ≥80% of measured areas with <1.0 slit/μm GBM, or segmental, 21% to 79% of evaluated areas with <1.0 slit/μm GBM.

Follow-up of El Salvador Biopsy Study

Seven of 8 patients from our previous kidney biopsy study in El Salvador[8] were followed up with blood and urine sampling 19 to 26 months after kidney biopsy. Samples were collected in May through July (ie, after harvest season). Four samples were transported to KUHL and analyzed for plasma creatinine (IDMS traceable), sodium, and potassium. Three samples were analyzed at Hospital Rosales, San Salvador, with a creatinine assay not traceable to IDMS. $eGFR_{cr}$ was calculated. When calculating change in $eGFR_{cr}$, baseline samples from the same laboratory were used. One patient had initiated hemodialysis therapy; creatinine analyzed before dialysis therapy initiation was used as follow-up. One patient was lost to follow-up.

Follow-up of Nicaragua Kidney Biopsy Study

Of 19 patients, 18 were followed up with blood and urine sampling 13 to 17 months after the biopsy. Samples were collected in June or October (ie, outside of harvest season). Samples were analyzed at KUHL for plasma creatinine (IDMS traceable), sodium, potassium, uric acid, and cystatin C (IDMS traceable) and urine albumin, creatinine, and α_1-microglobulin. $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$ were calculated. One patient (patient 14) was lost to follow-up.

Ethics Statement

Ethics committees in Stockholm, Sweden (2012/441-31/3; 2013/1225-32; 2015/849-32), at the National Autonomous University of Nicaragua at León (ACTA No 83, 2013; ACTA No 31, 2015), and at the Hospital Nacional Rosales, San Salvador, El Salvador (ACTA No 03, 2012; ACTA exp No 11, 2016) approved the study. All participants have given informed consent.

Statistical Analysis

In the follow-up study, simple linear regression was calculated to predict change in $eGFR_{cr}$ per year based on biochemical data at baseline. Morphology associations were calculated using unpaired *t* test comparing mean change in $eGFR_{cr}$ per year between groups with different morphology; patients with not representative biopsies (Nicaragua patients 4 and 11) were excluded.

RESULTS

Nicaragua Kidney Biopsy Study

There were 100 possible participants identified, 50 of whom matched the inclusion/exclusion criteria and were invited to participate. Twenty patients did not answer and 11 declined. Thus, 19 patients agreed and were included in the study. All participating patients had normal blood pressure 1 week prior to the biopsy.

Questionnaires

Exposure and clinical data are presented in Table 1. All patients had previously been working with sugarcane cultivation for 2.5 to 38 years. Work tasks were mainly cane cutting, irrigation, and seeding. Regular NSAID and acetaminophen use (defined as weekly use for ≥2 years) was reported from 32% and 53% of patients, respectively. There were 47% who reported no regular use of analgesic. Mean self-reported liquid intake for a workday was 9.0 (range, 3.4-15.1) L, and mean current daily liquid intake was 5.3 (range, 1-10) L. Water was the main liquid consumed (77% workdays, 72% of current liquids). Soda and fruit juice were 11% of the intake for workdays and 25% of the current intake. All but 2 patients used electrolyte solutions during the workday (mean, 11% of liquids consumed).

Blood Tests

Results of most analyses are presented in Table 2. Alanine aminotransferase level was elevated in 1 patient. Serum osmolality was elevated in 3 patients, at 304, 304, and 316 mOsm/L. Renin level was elevated in 8 patients; in these patients, mean sodium level was 136 (range, 135-137) mEq/L, 3 had hypokalemia, and all 8 had aldosterone levels within the reference range. In patient 1, aldosterone and aldosterone/renin levels were elevated, at 1,080 pmol/L and 60 pmol/mIU, respectively. All patients had plasma glucose, calcium, and phosphate levels within the reference ranges and had negative screening results for hepatitis B and C virus and human immunodeficiency virus (HIV). No antineutrophil cytoplasmic or anti-GBM antibodies were found. Complement levels showed no signs of activation. Antinuclear antibody screening with immunofluorescence was positive in patient 13; subsequent analysis for double-stranded DNA antibodies, centromere antibodies, and extractable nuclear antigens gave negative results.

Table 1. Nicaragua Biopsy Study: Clinical Characteristics and Questionnaire Data at Time of Kidney Biopsy

Characteristic	Value
Age, y	33 ± 8 (24-54)
BMI, kg/m^2	25 ± 4 (19-37)
Systolic BP, mm Hg	121 ± 8 (105-133)
Diastolic BP, mm Hg	75 ± 7 (56-88)
Kidney length, mm	91 ± 8 (74-104)
Duration of sugarcane work,[a] y	12 ± 8 (2.5-38)
Duration as sugarcane cutter, y	7 ± 7 (0-26)
Duration of agricultural work,[b] y	15 ± 10 (3-40)
Antihypertensive medicine	0 (0)
Antibiotics past 6 mo	0 (0)
Regular long-time NSAID use[c]	6 (32)
Current NSAID use	4 (21)
Current acetaminophen use	7 (37)
Herbal medicine use	1 (5)
Ever fainted at work	7 (37)
Exposure to chemicals/pesticides	7 (37)
Current smoking	0 (0)
Past smoking	6 (32)
Alcohol consumption	4 (21)

Note: n = 19. Values for categorical variables are given as count (percentage); for continuous variables, as mean ± standard deviation (range).

Abbreviations: BMI, body mass index; BP, blood pressure; NSAID, nonsteroidal anti-inflammatory drug.

[a]Defined as work at sugarcane plantation.

[b]Agricultural work including sugarcane work and other agricultural work.

[c]Use of 1 to 2 or more tablets per week during 2 years or longer.

Urine Tests

Results regarding urinary biomarkers and heavy metals are presented in Table 3. Two of the 4 patients with hypokalemia had urine potassium excretion > 40 mEq/L and all 4 hypokalemic patients had urine potassium-creatinine ratios > 1.5 mEq/mmol, indicating renal potassium losses.[16] In the 9 patients with low plasma sodium levels, mean urine sodium excretion was 88.6 ± 51.6 (range, 21-172) mEq/L. Dipstick showed no hematuria, leukocytes, or

Table 2. Nicaragua Biopsy Study: Blood Tests at Time of Biopsy

Variable	Mean ± SD (Range)
$eGFR_{cr-cys}$, mL/min/1.73 m^2	56 ± 18 (36-96)
$eGFR_{cr}$, mL/min/1.73 m^2	57 ± 18 (33-96)
$eGFR_{cys}$, mL/min/1.73 m^2	57 ± 20 (38-109)
Plasma	
Creatinine, mg/dL	1.66 ± 0.38 (1.04-2.51)
Cystatin C, mg/L	1.47 ± 0.31 (0.85-1.89)
Potassium, mEq/L	3.8 ± 0.6 (2.2-4.9)[a]
Sodium, mEq/L	137 ± 2 (133-139)[b]
Magnesium, mEq/L	1.41 ± 0.22 (1.02-1.64)[c]
Uric acid, mg/dL	7.45 ± 2.02 (4.44-12.02)[d]
Renin, mIU/L	68 ± 134 (18-650)
Aldosterone, pmol/L	265 ± 217 (86-1,080)
Aldosterone-renin ratio, pmol/mIU	9.5 ± 13.0 (0.3-60)
Albumin, g/dL	4.4 ± 0.3 (3.8-5.1)
Alanine aminotransferase, U/L	31 ± 33 (3-151)
Serum osmolality, mOsm/kg	296 ± 7 (288-316)

Note: n = 19. Reference values: eGFR, >90 mL/min/1.73 m^2; creatinine, <1.13 mg/dL; cystatin C, <0.99 mg/L; potassium, 3.5 to 5.0 mEq/L; sodium, 137 to 145 mEq/L; magnesium, 1.4 to 1.9 mEq/L; uric acid, 3.0 to 7.0 mg/dL; osmolality, 280 to 300 mOsm/kg; renin, 2.8 to 40 mIU/L; aldosterone, <650 pmol/L; albumin, 3.6 to 4.5 g/dL; alanine aminotransferase, <72 U/L; and aldosterone to renin ratio, <60 pmol/mIU. Conversion factors for units: creatinine in mg/dL to μmol/L, ×88.4; magnesium in mEq/L to mmol/L, ×0.5; uric acid in mg/dL to μmol/L, ×59.48; alanine aminotransferase in U/L to μkat/L, ×0.0167.

Abbreviations: $eGFR_{cr}$, estimated glomerular filtration rate based on creatinine; $eGFR_{cr-cys}$, estimated glomerular filtration rate based on creatinine and cystatin C; $eGFR_{cys}$, estimated glomerular filtration rate based on cystatin C; SD, standard deviation.

[a]Four (21%) patients had plasma potassium <3.5 mEq/L.
[b]Nine (47%) patients had plasma sodium <137 mEq/L.
[c]Seven (37%) patients had plasma magnesium <1.4 mEq/L.
[d]Eleven (58%) patients had plasma uric acid >7.0 mg/dL.

Table 3. Nicaragua Biopsy Study: Urine Test Results at Time of Biopsy

Urine Variable	Mean ± SD (Range)
Culture	All negative
Potassium, mEq/L	33 ± 17 (4-61)
Potassium-creatinine ratio, mEq/mmol	4.2 ± 1.3 (2.7-8.9)
Sodium, mEq/L	119 ± 59 (21-254)
pH	6.2 ± 0.4 (6-7)
24-h urine volume, L	3.1 ± 1.4 (1.3-6.7)[a]
ACR, mg/g	64 ± 194 (0.8-809)[b]
NAG-creatinine ratio, nkat/g	68 ± 53 (5-186)[c]
A1M-creatinine ratio, mg/g	27 ± 33 (3-141)[d]
Arsenic, μg/L	10.3 ± 6.6 (1.31-20.1)
Cadmium-creatinine ratio, μg/g	0.10 ± 0.09 (0.02-0.336)[e]
Mercury-creatinine ratio, μg/g	0.32 ± 0.25 (0.05-0.88)[f]
Lead-creatinine ratio, μg/g	0.98 ± 0.88 (0.15-2.93)[g]

Note: n = 19. Reference values: 24-hour urine volume, 0.5 to 3 L; ACR, <30 mg/g; NAG-creatinine ratio, <71 nkat/g; A1M-creatinine ratio, <6.2 mg/g; arsenic, <100 μg/L; cadmium-creatinine ratio, <2 μg/g; mercury-creatinine ratio, <5 μg/g; and lead-creatinine ratio, <50 μg/g. Conversion factors for units: ACR in mg/g to mg/mmol, ×0.113; NAG-creatinine ratio in nkat/g to nkat/mmol, ×0.113; A1M-creatinine ratio in mg/g to mg/mmol, ×0.113.

Abbreviations: A1M, α_1-microglobulin; ACR, albumin-creatinine ratio; NAG, *N*-acetyl-β-D-glucosaminidase; SD, standard deviation.

[a]Nine (47%) patients had 24-hour urine volume >3 L.
[b]Two (11%) patients had ACRs >30 mg/g.
[c]Eight (42%) patients had NAG-creatinine ratio > 71 nkat/g.
[d]Thirteen (68%) patients had A1M-creatinine ratios > 6.2 mg/g.
[e]Eight (42%) patients had no detectable urinary cadmium (ie, <0.05 μg/L).
[f]Ten (53%) patients had no detectable urinary mercury (ie, <0.2 μg/L).
[g]Ten (53%) patients had no detectable urinary lead (ie, <0.5 μg/L).

glucosuria; dipstick protein was increased in 2 patients (trace and 3+, respectively). Sediment showed no casts. Patients 7 and 15 had increased urinary albumin-creatinine ratios at 316 and 809 mg/g, respectively.

Kidney Biopsies

All 19 participants underwent kidney biopsy. One participant developed transient macroscopic hematuria; the other participants had no adverse events.

Light Microscopy and Immunofluorescence

Overview

There were 13 biopsy specimens that were representative; 3, marginally representative; and 3, not representative. Polarized light on paraffin-embedded tissue detected no crystals. No uric acid crystals were found using de Galantha staining. In 9 patients, frozen tissue was available for evaluation with polarized light; in 1 patient (patient 12), 1 round-shaped crystal was seen. Evaluation of the representative and marginally representative biopsy specimens (n = 16) are presented in the following text and Table 4. Information about the not representative biopsies (n = 3) is presented in Table S1.

Glomerular Changes

Specimens showed global glomerulosclerosis of varying proportions, 7% to 70%, and hypertrophy of the remaining glomeruli (Fig 1A). In patients 7 and 15, glomerular segmental sclerosis were found. Discrete mesangial matrix expansion was seen in most patients, but no mesangial cell proliferation. Focal wrinkling of the GBM and/or periglomerular fibrosis was seen in all but 1 patient (Fig 1A, C, and D). In 4 patients, no representative material for immunofluorescence was collected due to limited biopsy material. In the other patients, immunofluorescence showed no signs of immune complex disease.

Table 4. Nicaragua Biopsy Study: Light and Electron Microscopy Findings of Representative and Marginally Representative Biopsies

Microscopy Findings	Value
Light microscopy findings (n = 16)	
Glomerular changes (n = 16)	
Mean globally sclerosed glomeruli	38% ± 21% (7%-70%)
Percentage globally sclerosed glomeruli	
<25%	4 (25%)
25%-50%	7 (44%)
>50%	5 (31%)
Segmental scleroses	2 (12.5%)
Glomerular hypertrophy	
0	0 (0%)
1	0 (0%)
2	11 (69%)
3	5 (31%)
Wrinkled GBM/periglomerular fibrosis	
Yes	15 (94%)
No	1 (6%)
Tubulointerstitial changes (n = 16)	
Tubular atrophy[a]	
0	1 (6%)
1	13 (81%)
2	2 (13%)
3	0 (0%)
Interstitial fibrosis[a]	
0	1 (6%)
1F	8 (50%)
2F	7 (44%)
3F	0 (0%)
Interstitial inflammation[a]	
0	2 (13%)
1	12 (75%)
2	2 (13%)
3	0 (0%)
Vascular changes (n = 15)	
Intimal thickening	
0	11 (73%)
1	3 (20%)
2	1 (7%)
3	0 (0%)
Smooth muscle hyperplasia	
0	5 (33%)
1	6 (40%)
2	4 (27%)
3	0 (0%)
Electron microscopy findings (n = 16)	
GBM thickness, nm[b]	441 ± 63 (333-566)[c]
Podocyte foot processes, slits/μm GBM	1.0 ± 0.5 (0.1-1.7)
Podocyte foot process effacement[d]	
No (normal)	7 (44%)
Segmental effacement	4 (25%)
Widespread effacement	5 (31%)
Endothelial cells	
Normal	10 (62%)
Swollen	6 (38%)
Podocyte cytoplasm inclusions	
Yes	12 (75%)
No	4 (25%)
Immune complex deposits	
Yes	0 (0%)
No	16 (100%)

Note: Values are given as cases (percentage) or mean ± standard deviation (range). Mean number of glomeruli (n = 16): 17 ± 7 (7-28). Grading: 0, normal; 1, mild; 2, moderate; and 3, severe.

Abbreviations: F, focal; GBM, glomerular basement membrane.

[a]Grading of tubular atrophy, interstitial inflammation, and interstitial fibrosis is defined as 1 = mild, affecting <25% of area; 2 = moderate, affecting 26% to 50%; and 3 = severe, affecting >50%.

[b]GBM thickness normal range, 250 to 457 nm.

[c]Five (31%) patients had thickened GBM.

[d]Podocyte foot-process effacement defined as widespread, ≥80% of evaluated areas with <1.0 slit/μm GBM; segmental, 21%-79% of evaluated areas with <1.0 slit/μm GBM.

Tubulointerstitial Changes

Interstitial fibrosis was mild to moderate (Fig 1B) in most patients. Interstitial inflammation was generally mild, located in fibrotic areas, and consisting of lymphocytes. A few neutrophil granulocytes were found in tubuli of 2 patients. In 2 patients, a few eosinophils were found in the interstitium. Mild tubular atrophy was common (Fig 1C and D). Mild tubulitis was seen in 1 patient.

Vascular Changes

Arteries were found in all but 1 patient. Arterial changes were generally mild (Fig 1C and D; Table 4). Five specimens showed arteriolar hyalinosis (mild in 3 patients, moderate in 2).

Electron Microscopy

Results are presented in Tables 4 and S2. No immune deposits were found. Mild thickening of the GBM due to homogenous thickening of the lamina densa was seen in 5 patients. The podocytic cytoplasm often contained vacuoles (Fig 2A) or lipofuscin-like bodies (Fig 2B). Cell debris, probably derived from podocytes, was seen between capillary loops in Bowman's space in 3 patients (Fig 2C). The parietal epithelial cells of Bowman's capsule displayed no apparent pathology. Podocytic foot-process effacement was seen in 9 patients (Fig 2D). The endothelium was mostly normal, but in 6 patients, it was swollen at varying degrees (Fig 2D). In 4 patients, the swollen endothelium also showed reduced fenestration. Tubular structures showed varying

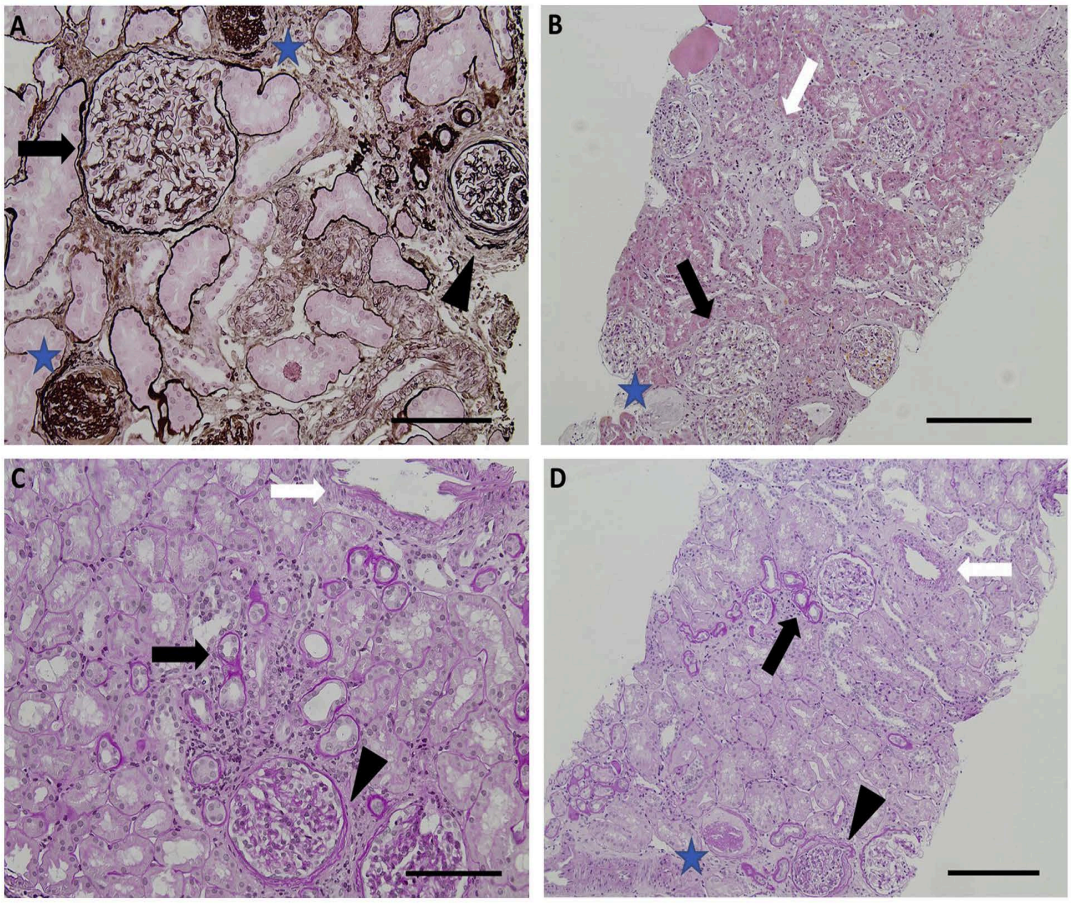

Figure 1. Light microscopy findings in kidney tissue from the Nicaragua biopsy study. Global glomerulosclerosis of varying degree (stars in A [periodic acid–Schiff (PAS)-methenamine staining from patient 1], B [Ladewig staining from patient 9], and D [PAS staining from patient 9]) and (A, B) moderate to severe glomerular hypertrophy (black arrow) were found in all patients. Signs of glomerular ischemia with thickening of Bowman's capsule or wrinkling of capillary walls (arrowheads in A, C [PAS staining from patient 6], and D) were found in all but 1 patient. (B) Mild to moderate interstitial fibrosis (white arrow) and (C, D) tubular atrophy of varying degree (black arrows) were seen in most patients. (C, D) Arteries were in most cases normal (white arrows) or only mildly changed. Scale bars = (A, C) 100 μm, (B, D) 200 μm.

degrees of tubular atrophy, but otherwise no apparent pathology.

Follow-up of El Salvador and Nicaragua Biopsy Studies

In total, 25 of 27 participants in the 2 biopsy studies were included in the follow-up, and they had a mean change in $eGFR_{cr}$ per year of -4.4 ± 8.4 (range, -27.7 to 10.2) mL/min/1.73 m^2.

El Salvador Follow-up

Seven of 8 patients were followed up 19 to 26 months after kidney biopsy (Table 5). One patient had initiated hemodialysis therapy after 19 months (with $eGFR_{cys}$ of 9 mL/min/1.73 m^2 3 days before dialysis therapy initiation). Urine samples were available for 5 patients; 2 had increased urine albumin-creatinine ratios (2,458 and 45 mg/g). Two patients were working with sugarcane, 4 with other agricultural work, and 1 was unemployed.

Nicaragua Follow-up

Eighteen of 19 patients were followed up 13 to 17 months after kidney biopsy (Table 5). Urine albumin-creatinine ratios were increased in patients 7 and 19 (462 and 2,270 mg/g). Ten patients were unemployed or on pension; 2, construction workers; 1, an agricultural worker (not sugarcane); 1, a baker; 1, a mechanic; and 3, in non–physically strenuous professions.

Possible Predictors of Decrease in eGFR

Simple linear regression was performed to predict change in $eGFR_{cr}$ based on various biochemical data at the time of the biopsy (t_0); results are presented in Table S3. A significant correlation was seen between plasma sodium level and change in $eGFR_{cr}$ per year when combining both cohorts (El Salvador and Nicaragua, n = 25). Introducing baseline eGFR as a covariate in the equation did not significantly ($P = 0.7$) influence the decline in eGFR related to plasma sodium

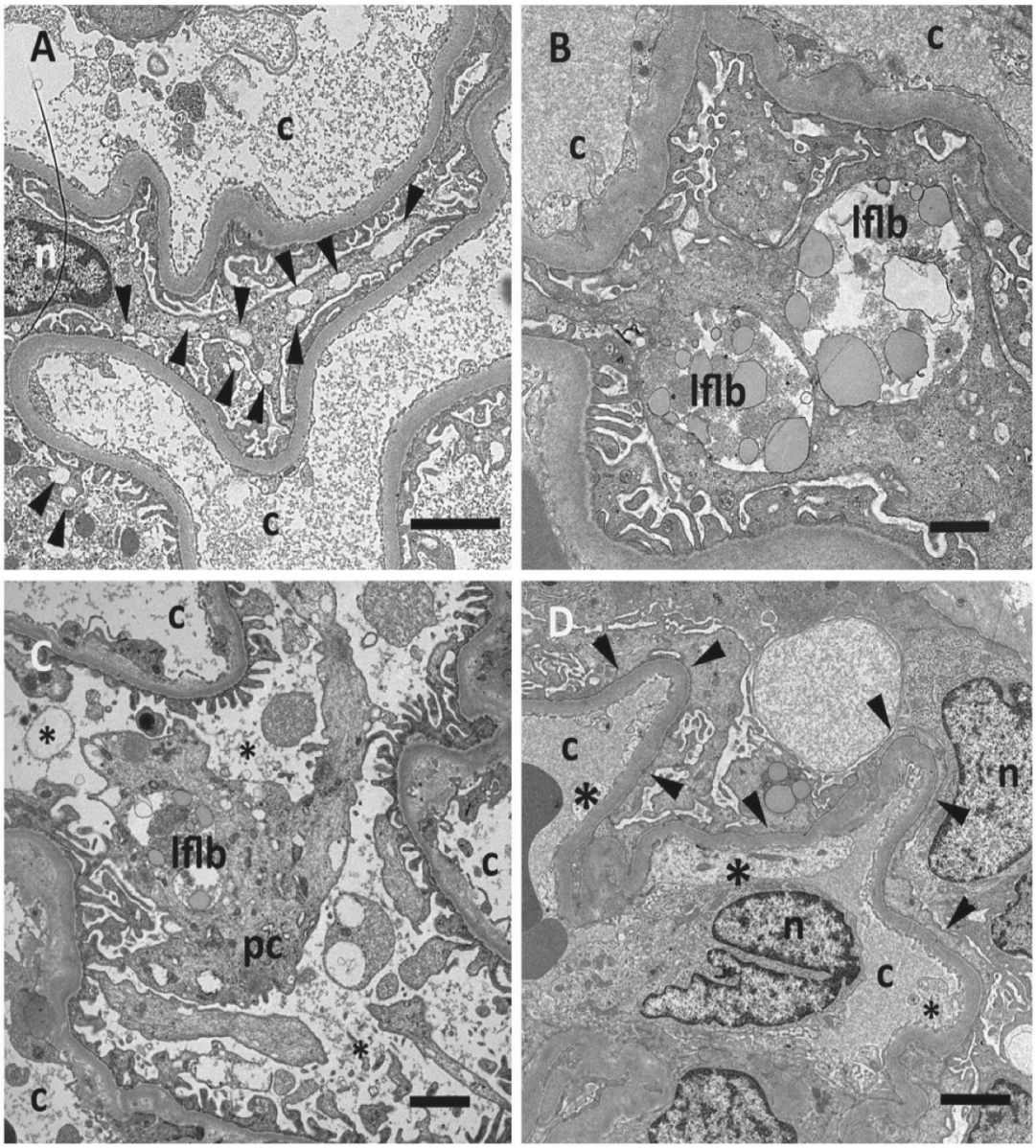

Figure 2. (A) Transmission electron microscopy image from patient 10 shows cytoplasm of a podocyte (pc) containing vacuoles (arrowhead). (B) Image from patient 17 shows lipofuscin-like bodies (lflb) in cytoplasm of a podocyte. (C) Image from patient 6 shows cell debris in Bowman's space (*), probably derived from podocytes because it was mostly found within the glomerular tuft between capillary loops and Bowman's capsular epithelium showed no apparent pathology. (D) Image from patient 17 shows widespread foot-process effacement (arrowhead) and a focally swollen endothelium (*). Abbreviations: c, capillary space; n, nucleus. Scale bars = (A) 5 μm, (B-D) 1 μm.

level ($P = 0.02$). Therefore, baseline eGFR was not used as a covariate in any of the calculations in Table S3.

Patients with severe glomerular enlargement had a significantly larger decrease in $eGFR_{cr}$ compared with patients with mild to moderate enlargement (Table S4).

DISCUSSION

This unique study describes the renal morphology and biochemical characteristics of 19 patients with MeN in Nicaragua and also presents 1- to 2-year follow-up data from this cohort, as well as from our previous biopsy study of 8 patients with MeN in El Salvador.

The renal morphology shows glomerulosclerosis of varying degrees, glomerular hypertrophy, and signs of chronic glomerular ischemia, together with mild to moderate chronic tubulointerstitial damage. The morphology is very similar to findings in our previous study of MeN cases.[8]

Table 5. Follow-up Plasma and Urine Data From El Salvador and Nicaragua Kidney Biopsy Studies

	El Salvador Biopsy (n = 7)	Nicaragua Biopsy (n = 18)
Postbiopsy follow-up time, mo	25 ± 3 (19-27)	14 ± 2 (13-17)
$eGFR_{cr}$, mL/min/1.73 m^2	45 ± 25 (9 to 76)	52 ± 20 (20 to 87)
$\Delta eGFR_{cr}$, mL/min/1.73 m^2 per y	−3.6 ± 5.2 (−13.3 to 2.8)	−5.3 ± 9.2 (−27.7 to 10.2)
$eGFR_{cr-cys}$, mL/min/1.73 m^2		48 ± 20 (18 to 85)
$\Delta eGFR_{cr-cys}$, mL/min/1.73 m^2 per y		−7.0 ± 8.2 (−24.9 to 6.5)
Plasma		
Potassium, mEq/L	3.3 ± 0.7 (1.9 to 4)[a]	4.3 ± 0.6 (2.8 to 4.8)[b]
Sodium, mEq/L	132 ± 4 (126 to 136)	138 ± 1 (135 to 140)[b]
Uric acid, mg/dL	—	7.30 ± 2.52 (4.12 to 12.19)[b]
Urine		
ACR, mg/g	508 ± 1,091 (4 to 2,458)[a]	155 ± 539 (1 to 2,270)[b]
NAG:Cr ratio, nkat/g	108 ± 46 (53 to 168)[a]	
A1M:Cr ratio, mg/g	-	24 ± 34 (4.5 to 144)[b]

Note: Values are given as mean ± standard deviation (range). Reference values: potassium, 3.5 to 5.0 mEq/L; sodium, 137 to 145 mEq/L; uric acid, 3.0 to 7.0 mg/dL; ACR, <30 mg/g; NAG:Cr ratio, <71 nkat/g; A1M/Cr ratio, <6.2 mg/g. To convert uric acid in mg/dL to μmol/L, ×59.48.

Abbreviations: A1M, α_1-microglobulin; ACR, albumin-creatinine ratio; Cr, creatinine; $eGFR_{cr}$, estimated glomerular filtration rate based on creatinine; $eGFR_{cr-cys}$, estimated glomerular filtration rate based on creatinine and cystatin C; NAG, *N*-acetyl-β-D-glucosaminidase.

[a]Potassium was <3.5 mEq/L in 4 patients; ACR, >30 mg/g in 2 patients; and NAG:Cr ratio, >71 nkat/g in 4 patients.

[b]Potassium was <3.5 mEq/L in 2 patients; sodium, <137 mEq/L in 4 patients; uric acid, >7.0 mg/dL in 7 patients; ACR, >30 mg/g in 2 patients; and A1M:Cr ratio, >6.2 mg/g in 11 patients.

We used similar inclusion and exclusion criteria as in our previous biopsy study, but this study included twice as many patients, enabling a more detailed description of the disease. Furthermore, $eGFR_{cr-cys}$ in this study is higher (mean, 56 compared to 42 mL/min/1.73 m^2) and participants are younger (mean, 33 compared to 44 years), thus reducing possible age-related morphologic changes and describing earlier lesions in MeN.

Chronic glomerular changes were seen in all patients. Glomerular hypertrophy was present in all patients and could be the result of compensatory mechanisms due to nephron loss/low nephron count or chronic ischemia.[17,18] In all but one patient, wrinkling of the GBM and/or periglomerular fibrosis was seen. These changes, indicating glomerular ischemia, are often found in nephrosclerosis,[19] but neither hypertension nor vascular changes consistent with hypertension were found. Immune complex glomerulonephritis could be excluded in all representative biopsies by immunofluorescence or electron microscopy.

The chronic tubulointerstitial damage was in most cases mild and in some cases was moderate. Signs of tubular damage were also evident in urine, in which NAG or α_1-microglobulin levels often were elevated. A few granulocytes were found in tubuli of 2 patients, but because urine culture results were negative, acute pyelonephritis is unlikely. Chronic pyelonephritis is more difficult to exclude. However, in a previous report, urine cultures from 103 sugarcane workers all gave negative results, indicating that infections are uncommon.[20]

Podocyte foot-process effacement was found in 9 of 16 patients, but was not correlated to albuminuria because only 1 of these patients displayed albuminuria. Other signs of podocytic injury, including podocyte debris and lipofuscin-like bodies, were also found. Whether there is primary damage to the podocytes or secondary damage due to glomerular hypertrophy is unclear.

In 2014, López-Marín et al[21] published a study of kidney biopsies from 46 Salvadoran patients with eGFRs of 30 to 89 mL/min/1.73 m^2. They concluded that their main finding was "chronic tubulointerstitial nephropathy" with subsequent glomerular and vascular damage. However, they also reported that 67% of the men in the study had >25% global glomerulosclerosis and 83% of the men had <25% tubular atrophy (ie, results similar to our findings).

Based on our previous study, others have described the morphology of MeN as "chronic tubulointerstitial nephropathy with secondary glomerular damage."[22] We do not agree with this phrase because we would describe the morphology as a combination of chronic glomerular and tubulointerstitial changes for which the mechanism has to be elucidated. That tubulointerstitial damage can cause glomerular damage or vice versa is well known.[23] In our experience, the ratio between the glomerular and cortical tubulointerstitial damages found in our studies of MeN suggest that the tubulointerstitial damage alone might not be enough to explain the glomerular changes.

The finding of low plasma sodium, potassium, and magnesium levels in many patients (Tables 2 and 5) is striking. Spot urine samples suggest that the hypokalemic patients in our Nicaraguan cohort have renal potassium losses, a finding that also has been reported from another MeN study.[24] Interestingly, when combining data from both biopsy cohorts, low sodium at baseline was correlated with a higher progression rate (Table S3). To establish if this is a correlation or causation, further studied in larger cohorts are needed. Hyponatremia correlated to sugarcane work,[25] as well as extreme exercise,[26,27] has been described, but our study was performed outside of harvest season, indicating a chronic state independent from the extreme conditions during sugarcane harvest. The participants' large urine volumes (mean, 3.1 L/24 h) are noteworthy. Excessive water intake may contribute to the hyponatremia in some patients. We have previously argued for possible renin-angiotensin-aldosterone system activation as one of the mechanisms behind the glomerular ischemia and hypokalemia seen in MeN.[8] Our findings further support the hypothesis that chronic sodium depletion may stimulate the renin-angiotensin-aldosterone system, whereby angiotensin II would cause glomerular ischemia by constriction of glomerular capillaries and aldosterone would cause hypokalemia. Plasma renin level was increased in 8 patients, who also were low or in the lower reference range for plasma sodium. However, aldosterone levels were in most cases within the reference ranges, making the data difficult to interpret. Analysis of renin, angiotensin II, and aldosterone during harvest would be of great interest.

To our knowledge, this is the first report presenting follow-up data from patients with kidney biopsy–verified MeN. Our data show a mean decline in $eGFR_{cr}$ of 4.4 mL/min/1.73 m^2 per year. However, individual variation in change in eGFR over time was great (Table 5).

When comparing kidney morphology with progression rate, we found that patients with severe glomerular hypertrophy had a worse prognosis (Table S4). This finding needs to be confirmed in larger studies.

The cause of MeN is not fully understood, but the leading hypothesis is occupational heat exposure with repeated episodes of volume and salt depletion.[6,28,29] That volume depletion can lead to acute kidney injury (AKI) is known, and in recent years, AKI has been found to be a risk factor for CKD development.[30,31] Three studies of sugarcane workers report increasing serum creatinine levels of about 10% to 20% after a workday,[25,29,32] supporting the hypothesis that repeated AKI caused by extreme working conditions may be the major cause of MeN.

Interestingly, a morphologic picture similar to that of MeN has been described in rats developing CKD after AKI.[33-35] Kidney morphology 9 months after ischemic AKI is described as glomerular hypertrophy, glomerulosclerosis, podocyte foot-process effacement, and tubulointerstitial damage. Furthermore, rats developing CKD after AKI were normotensive, another similarity with patients with MeN. Spironolactone and losartan treatment prevented the development of CKD, suggesting that these agents, affecting the renin-angiotensin-aldosterone system, are of importance in the pathophysiology.

A possible mechanism for developing CKD due to repeated dehydration involving fructose metabolism in tubuli has been presented in a study of mice.[36] The authors suggest the main pathologic mechanism to be tubular damage caused by end products in the fructose metabolism (inflammatory mediators, oxidants, and uric acid). Hyperuricemia and hyperuricosuria have also been hypothesized as possible mechanisms.[37] In our study, we did not find support for uric acid nephropathy; no uric acid crystals or lesions related to hyperuricemia were seen. However, we cannot exclude the possibility because biopsies were not performed during dehydration.

The role of NSAIDs in the development of MeN has been discussed.[1,38] In the present study, most Nicaraguan participants did not use NSAIDs. However, the pharmacodynamic properties of NSAIDs, reducing renal blood flow, are probably harmful in MeN, in which glomerular ischemia is already present.

Heavy metals have been raised as possible cofactors in MeN development.[39] In this study, we could not find evidence for this because all values were below toxic levels.[12,13,40]

In recent years, the resemblance between MeN and CKD of unknown origin affecting rural inhabitants in Sri Lanka has been discussed.[38,41] Tubulointerstitial damage has been described as the main lesion in kidney biopsy specimens, but glomerulosclerosis, glomerular enlargement, and thickening of Bowman's capsule have also been reported,[42,43] indicating a resemblance with MeN. Most biopsy studies in Sri Lanka have included participants by screening for proteinuria, and this makes comparison difficult because patients with MeN often lack proteinuria.

In summary, we propose that the findings in our study are compatible with the hypothesis that MeN is caused by recurrent kidney injury due to occupational heat stress with subsequent volume and salt depletion. Kidney function often deteriorates in patients with MeN, and risk factors for this should be further studied to enable preventive strategies. Comparative studies with CKD of unknown cause in other countries are needed to elucidate whether MeN might be a global disease, accelerated by global warming.

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Contributions: Research idea and study design: JW, MGQ, MH, ZT, AA, C-GE, AW; data acquisition: MGQ, MH, ZT, AA; tissue preparation: KH, AR; data analysis/interpretation: JW, MGQ, ZT, KH, MS, C-GE, AW; statistical analysis: JW, C-GE; supervision or mentorship: AW, C-GE, KH, AA. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. AW takes responsibility that this study has been reported honestly, accurately, and transparently; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

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SUPPLEMENTARY MATERIAL

Table S1: Nicaragua biopsy study, light microscopy findings of not-representative biopsies.

Table S2: Nicaragua biopsy study, electron microscopy findings.

Table S3: Biochemical predictors of change in eGFR.

Table S4: Morphology predictors of change in eGFR per year.

Item S1: Tissue preparation of kidney biopsies.

Note: The supplementary material accompanying this article (http://dx.doi.org/10.1053/j.ajkd.2016.10.036) is available at www.ajkd.org

REFERENCES

1. Wesseling C, Crowe J, Hogstedt C, Jakobsson K, Lucas R, Wegman D, eds. *Mesoamerican Nephropathy: Report From the First International Research Workshop on MeN*. Heredia, Costa Rica: SALTRA/IRET-UNA; 2013. http://www.saltra.una.ac.cr/index.php?option=com_content&view=article&id=109&Itemid=269. Accessed August 8, 2016.

2. Wegman D, Crowe J, Hogstedt C, Jakobsson K, Wesseling C, eds. *Mesoamerican Nephropathy: Report From the Second International Research Workshop on MeN*. Heredia, Costa Rica: SALTRA/IRET-UNA; 2016. http://www.saltra.una.ac.cr/index.php?option=com_content&view=article&id=109&Itemid=269. Accessed August 8, 2016.

3. UpToDate. Mesoamerican nephropathy. http://www.uptodate.com/contents/mesoamerican-nephropathy. Accessed August 5, 2016.

4. Torres C, Aragon A, Gonzalez M, et al. Decreased kidney function of unknown cause in Nicaragua: a community-based survey. *Am J Kidney Dis*. 2010;55(3):485-496.

5. Peraza S, Wesseling C, Aragon A, et al. Decreased kidney function among agricultural workers in El Salvador. *Am J Kidney Dis*. 2012;59(4):531-540.

6. Wesseling C, Crowe J, Hogstedt C, Jakobsson K, Lucas R, Wegman DH. Resolving the enigma of the Mesoamerican nephropathy: a research workshop summary. *Am J Kidney Dis*. 2013;63(3):396-404.

7. Johnson RJ, Sanchez-Lozada LG. Chronic kidney disease: Mesoamerican nephropathy-new clues to the cause. *Nat Rev Nephrol*. 2013;9(10):560-561.

8. Wijkstrom J, Leiva R, Elinder CG, et al. Clinical and pathological characterization of Mesoamerican nephropathy: a new kidney disease in Central America. *Am J Kidney Dis*. 2013;62(5):908-918.

9. US Environmental Protection Agency (EPA). Method 200.8: Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry, Revision 5.4. 1994. https://www.epa.gov/sites/production/files/2015-06/documents/epa-200.8.pdf. Accessed August 5, 2016.

10. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604-612.

11. Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med*. 2012;367(1):20-29.

12. Dabt DEK, Berger-Ritchie J, McMillin GA. Testing for toxic elements: a focus on arsenic, cadmium, lead, and mercury. *Lab Med*. 2011;42(12):735-742.

13. US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. ToxGuide™ for Arsenic, October 2007. http://www.atsdr.cdc.gov/toxguides/toxguide-2.pdf. Accessed August 5, 2016.

14. Luna LG; Armed Forces Institute of Pathology (U.S.). *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3d ed. New York, NY: Blakiston Division; 1968.

15. Solez K, Colvin RB, Racusen LC, et al. Banff 07 classification of renal allograft pathology: updates and future directions. *Am J Transplant*. 2008;8(4):753-760.

16. UpToDate. Evaluation of the adult patient with hypokalemia. http://www.uptodate.com/contents/evaluation-of-the-adult-patient-with-hypokalemia. Accessed August 5, 2016.

17. Fogo A, Ichikawa I. Evidence for a pathogenic linkage between glomerular hypertrophy and sclerosis. *Am J Kidney Dis*. 1991;17(6):666-669.

18. Heyman SN, Khamaisi M, Rosen S, Rosenberger C. Renal parenchymal hypoxia, hypoxia response and the progression of chronic kidney disease. *Am J Nephrol*. 2008;28(6):998-1006.

19. Colvin RB. *Diagnostic Pathology. Kidney Diseases*. 1st ed. Salt Lake City, UT: Amirsys; 2011.

20. McClean M, Amador J, Laws R, et al. Biological sampling report: investigating biomarkers of kidney injury and chronic kidney disease among workers in Western Nicaragua, April 26, 2012. http://www.cao-ombudsman.org/cases/document-links/links-82.aspx. Accessed August 5, 2016.

21. Lopez-Marin L, Chavez Y, Garcia XA, et al. Histopathology of chronic kidney disease of unknown etiology in

Salvadoran agricultural communities. *MEDICC Rev*. 2014;16(2):49-54.

22. Roncal-Jimenez CA, Garcia-Trabanino R, Wesseling C, Johnson RJ. Mesoamerican nephropathy or global warming nephropathy? *Blood Purif*. 2016;41(1-3):135-138.

23. Kriz W, LeHir M. Pathways to nephron loss starting from glomerular diseases-insights from animal models. *Kidney Int*. 2005;67(2):404-419.

24. Herrera R, Orantes CM, Almaguer M, et al. Clinical characteristics of chronic kidney disease of nontraditional causes in Salvadoran farming communities. *MEDICC Rev*. 2014;16(2):39-48.

25. Paula Santos U, Zanetta DM, Terra-Filho M, Burdmann EA. Burnt sugarcane harvesting is associated with acute renal dysfunction. *Kidney Int*. 2014;87(4):792-799.

26. Irving RA, Noakes TD, Buck R, et al. Evaluation of renal function and fluid homeostasis during recovery from exercise-induced hyponatremia. *J Appl Physiol*. 1991;70(1):342-348.

27. Speedy DB, Noakes TD, Rogers IR, et al. Hyponatremia in ultradistance triathletes. *Med Sci Sports Exerc*. 1999;31(6):809-815.

28. Correa-Rotter R, Wesseling C, Johnson RJ. CKD of unknown origin in Central America: the case for a Mesoamerican nephropathy. *Am J Kidney Dis*. 2014;63(3):506-520.

29. Garcia-Trabanino R, Jarquin E, Wesseling C, et al. Heat stress, dehydration, and kidney function in sugarcane cutters in El Salvador - a cross-shift study of workers at risk of Mesoamerican nephropathy. *Environ Res*. 2015;142:746-755.

30. Coca SG, Singanamala S, Parikh CR. Chronic kidney disease after acute kidney injury: a systematic review and meta-analysis. *Kidney Int*. 2012;81(5):442-448.

31. Chawla LS, Eggers PW, Star RA, Kimmel PL. Acute kidney injury and chronic kidney disease as interconnected syndromes. *N Engl J Med*. 2014;371(1):58-66.

32. Wesseling C, Aragon A, Gonzalez M, et al. Kidney function in sugarcane cutters in Nicaragua - a longitudinal study of workers at risk of Mesoamerican nephropathy. *Environ Res*. 2016;147:125-132.

33. Barrera-Chimal J, Perez-Villalva R, Rodriguez-Romo R, et al. Spironolactone prevents chronic kidney disease caused by ischemic acute kidney injury. *Kidney Int*. 2013;83(1):93-103.

34. Barrera-Chimal J, Perez-Villalva R, Ortega JA, et al. Mild ischemic injury leads to long-term alterations in the kidney: amelioration by spironolactone administration. *Int J Biol Sci*. 2015;11(8):892-900.

35. Rodriguez-Romo R, Benitez K, Barrera-Chimal J, et al. AT1 receptor antagonism before ischemia prevents the transition of acute kidney injury to chronic kidney disease. *Kidney Int*. 2016;89(2):363-373.

36. Jimenez CAR, Ishimoto T, Lanaspa MA, et al. Fructokinase activity mediates dehydration-induced renal injury. *Kidney Int*. 2014;86(2):294-302.

37. Roncal-Jimenez C, Garcia-Trabanino R, Barregard L, et al. Heat stress nephropathy from exercise-induced uric acid crystalluria: a perspective on Mesoamerican nephropathy. *Am J Kidney Dis*. 2016;67(1):20-30.

38. Weiner DE, McClean MD, Kaufman JS, Brooks DR. The Central American epidemic of CKD. *Clin J Am Soc Nephrol*. 2013;8(3):504-511.

39. Said S, Hernandez GT. Environmental exposures, socioeconomics, disparities, and the kidneys. *Adv Chronic Kidney Dis*. 2015;22(1):39-45.

40. Nordberg G, Fowler BA, Nordberg M. *Handbook on the Toxicology of Metals*. 4th ed. London, UK: Elsevier; 2015.

41. Wanigasuriya KP, Peiris-John RJ, Wickremasinghe R, Hittarage A. Chronic renal failure in North Central Province of Sri Lanka: an environmentally induced disease. *Trans R Soc Trop Med Hyg*. 2007;101(10):1013-1017.

42. Athuraliya NT, Abeysekera TD, Amerasinghe PH, et al. Uncertain etiologies of proteinuric-chronic kidney disease in rural Sri Lanka. *Kidney Int*. 2011;80(11):1212-1221.

43. Nanayakkara S, Komiya T, Ratnatunga N, et al. Tubulointerstitial damage as the major pathological lesion in endemic chronic kidney disease among farmers in North Central Province of Sri Lanka. *Environ Health Prev Med*. 2012;17(3):213-221.

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Heat stress, hydration and uric acid: a cross-sectional study in workers of three occupations in a hotspot of Mesoamerican nephropathy in Nicaragua

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ABSTRACT

Objectives: To study Mesoamerican nephropathy (MeN) and its risk factors in three hot occupations.

Design: Cross-sectional.

Setting: Chinandega and León municipalities, a MeN hotspot on the Nicaraguan Pacific coast, January–February 2013.

Participants: 194 male workers aged 17–39 years: 86 sugarcane cutters, 56 construction workers, 52 small-scale farmers.

Outcome measures: (1) Differences between the three occupational groups in prevalences/levels of socioeconomic, occupational, lifestyle and health risk factors for chronic kidney disease (CKD) and in biomarkers of kidney function and hydration; (2) differences in prevalences/levels of CKD risk factors between workers with reduced estimated glomerular filtration rate ($eGFR_{CKD\text{-}EPI}$ <80 mL/min/1.73 m^2) and workers with normal kidney function ($eGFR_{CKD\text{-}EPI}$ ≥80 mL/min/1.73 m^2).

Results: Sugarcane cutters were more exposed to heat and consumed more fluid on workdays and had less obesity, lower blood sugar, lower blood pressure and a better lipid profile. Reduced eGFR occurred in 16%, 9% and 2% of sugarcane cutters, construction workers and farmers, respectively (trend cane > construction > farming, p=0.003). Significant trends (cane > construction > farming) were also observed for high serum urea nitrogen (blood urea nitrogen (BUN) >20 mg/dL), high serum creatinine (SCr >1.2 mg/dL), low urinary pH (≤5.5) and high BUN/SCr ratio (>20) but not for high urinary specific gravity (≥1.030). Sugarcane cutters also more often had proteinuria and blood and leucocytes in the urine. Workers with eGFR <80 mL/min/1.73 m^2 reported a higher intake of water and lower intake of sugary beverages. Serum uric acid levels related strongly and inversely to eGFR levels (adj β −10.4 mL/min/1.73 m^2, 95% CI −12.2 to −8.5, p<0.001). No associations were observed for other metabolic risk factors, pesticides, non-steroidal anti-inflammatory drugs or alcohol. Among cane cutters, consumption of electrolyte hydration solution appeared preventive (adj β 8.1 mL/min/1.73 m^2, p=0.09).

Conclusions: Heat stress, dehydration and kidney dysfunction were most common among sugarcane cutters. Kidney dysfunction also occurred to a lesser extent among construction workers, but hardly at all among small-scale farmers. High serum uric acid was associated with reduced kidney function.

Strengths and limitations of this study

- The study provides a detailed description of exposures to potential risk factors for Mesoamerican nephropathy (MeN) among workers in three occupations of special interest: subsistence farmers, construction workers and sugarcane cutters.
- The study established the prevalence of kidney dysfunction and dehydration among workers in these three distinct occupations at risk for MeN.
- The cross-sectional design limits causal interpretations about associations between the potential risk factors and the markers of kidney function, but the study provides clues for aetiology and possible pathways of kidney injury.
- Most exposures to risk factors are self-reported, but much attention was paid to the quality of the questionnaires.

INTRODUCTION

Mesoamerican nephropathy (MeN), an epidemic of chronic kidney disease (CKD), is a chronic tubulointerstitial disease unrelated to traditional CKD risk factors, affecting predominantly young male workers in Pacific coastal communities of Central America and possibly southern Mexico.[1–4] Several tens of thousands of people have died of this

disease.[3] Although MeN is often described as an epidemic of agricultural workers,[1 5–8] in Central America sugarcane workers are clearly the most affected population.[1 9 10]

A consistent risk factor for MeN appears to be heavy manual labour in extreme heat.[1] Manual sugarcane cutters exert substantial amounts of energy, often in environmental temperatures over 35°C and high humidity.[11–13] Besides heat stress, some sugarcane workers are also exposed to pesticides, either at sugarcane plantations or while labouring in other crops.[11 14] Consumption of non-steroidal anti-inflammatory drugs (NSAIDs) to manage muscle pain is common.[15] Exposure to heavy metals may occur through contaminated pesticide formulations and fertilisers, as has been shown in Sri Lanka,[16] contaminated drinking water,[17] or even during burning of the cane.[18] Overall, exposure of sugarcane workers to different potential CKD risk factors has not been described in detail.

A leading hypothesis is that recurrent dehydration, possibly in combination with exposure to other agents (eg, NSAIDs, heavy metals, agrochemicals, high fructose intake) may be a driving factor.[1 4] Animal experiments have shown that dehydration and hyperosmolarity may induce tubular injury via activation of the polyol-fructokinase pathway in the kidney.[19] Recently, a mechanism of hyperuricaemia and cyclical uricosuria associated with volume loss and dehydration has also been proposed.[20 21]

Studies suggest that MeN may also occur among miners and construction workers,[5 22] cotton workers[23] and subsistence farmers.[6] However, these cross-sectional data mostly consider current occupation and are therefore not conclusive. Cane cutting is seasonal and many sugarcane workers are also subsistence farmers or work in construction. Contrary to contracted workers, independent small-scale farmers have control over their work hours and are able to avoid the hottest temperatures. Prevalence studies have been recommended to assess exposure to CKD risk factors and kidney dysfunction in different occupations.[1]

The aim of this study was to compare the prevalence of a range of potential CKD risk factors among sugarcane cutters, construction workers and small-scale farmers labouring in the same hot environment, along with biomarkers of hydration and kidney function. We hypothesise that sugarcane cutters experience more heat stress, more dehydration and more signs of kidney dysfunction than small-scale farmers, with construction workers somewhere in between.

METHODS

Study population and recruitment

This is a cross-sectional study. We recruited 194 male workers aged 17–39 years, all living in the municipalities of Chinandega and León in the Pacific region of Nicaragua, a major epicentre for the MeN epidemic. Of these, 86 were sugarcane cutters, 56 construction workers and 52 small-scale farmers. Cane cutters from several sugarcane villages were recruited with the help of community leaders; a trade union assisted in recruiting construction workers employed by private companies at three construction sites; and a rural farmer association helped to recruit associated farmers dedicated full time to the cultivation of subsistence crops. The response rate was 86% among cane cutters and there were no refusals among construction workers and farmers.

The study was approved by the Ethical Review Board of UNAN-León, Nicaragua. All participants provided written informed consent.

Data collection

Data were collected for sugarcane cutters during January 2013, 2 months after the sugarcane harvest started, and during February 2013 for construction workers and farmers under similar climatic conditions. In each of the sugarcane and farming villages a well-known public place was selected as the data collection station; construction workers were evaluated at their work site. Data collection started between 05:30 and 06:00 hours on the morning after a workday and blood and urine samples were collected after overnight fasting.

Medical measurements and biological samples

Blood pressure was measured with a calibrated digital sphygmomanometer with the participant seated after resting for 10 min. Weight was measured with a calibrated digital flat mobile scale and height with a foldable stadiometer. Certified technicians collected blood samples in vacuum tubes for centrifugation and serum separation and in a tube with anticoagulant for blood cell count. Samples without coagulant were centrifuged on the spot at 3500 rpm for 10 min at room temperature. All samples were placed on ice and transported the same day to the laboratory at the Research Center on Health, Work and Environment (CISTA) at UNAN-León where haematocrit and haemoglobin were determined with a Mindray 2300 haematology analyser and the serum samples were frozen at −80°C. After finalising all data collection, serum samples were transported to the National Diagnostic and Reference Center of the Ministry of Health (CNDR-MINSA) of Nicaragua, which takes part in an international interlaboratory quality control programme. Samples were analysed with Cobas Integra 400, an automated equipment which uses a photometric test to determine levels of serum glucose, lipid profile, serum uric acid (S-UA) and blood urea nitrogen (BUN) and a Jaffe compensated method for quantification of serum creatinine (SCr). SCr was calibrated against IDMS-traceable creatinine. Blind spiked and duplicate blood samples from each tenth participant were in 95% within 1 SD. A urinalysis dipstick was performed on a spot morning sample using a Bayer Clinitek 50 Urine Chemistry Analyser with Multistix 10SG reagent strips (Siemens Diagnostics, USA) with

semi-quantitative measurements of protein (≥30–<300 mg/dL and ≥300 mg/dL, glucose (positive at ≥100 mg/dL), urinary specific gravity (USG) (1.000–1.030), pH (5.0–8.5), blood (+ to +++), nitrite (positive), leucocyte esterase (+ to +++), bilirubin (+ to +++), ketone (≥5 mg/dL) and urobilinogen (≥2 Ehrlich units).

Questionnaires

Questionnaires were applied by trained interviewers with courses on bioethics and good clinical practices. A questionnaire on work and health obtained data on demographics and employment (age, education, drinking water source, income, type of contract, sub-employment, social security), lifestyle (smoking, alcohol, drugs, fluid intake on non-working days), health (medically diagnosed diseases, nephrotoxic medications), work history (industry, job titles, job duration, crops, pesticides) and occupational heat stress determinants (shift duration, breaks, shadow, work speed, heavy loads; in addition, for sugarcane workers, incentives to cut more cane and hours between cane burning and entering the field). This questionnaire was developed based on versions used in previous studies in the region.[5 23 24]

A second questionnaire, developed at the National Institute of Public Health in Mexico, obtained data on the types and amounts of fluids and food items consumed during the day (always a workday) before the interview. The amount of fructose contained in the food and drinking items was estimated based on a fructose calculation list of the Mexican questionnaire[25] and the USDA National Nutrient Database for Standard Reference for items not included in the Mexican questionnaire.[26]

Statistical analysis

Data were analysed with SPSS Statistics 20. Glomerular filtration rate estimated by the CKD-EPI equation ($eGFR_{CKD\text{-}EPI}$) was the main outcome measure, categorised into <80 mL/min/1.73 m^2 and ≥80 mL/min/1.73 m^2. This cut-off point was chosen instead of the traditional<60 because too few workers had estimated glomerular filtration rate (eGFR) <60. Prevalences of high BUN (>20 mg/dL), high SCr (>1.2 mg/dL), high S-UA (>7.2 mg/dL) and protein >30 mg/dL, blood, nitrites or leucocytes in urine were secondary measures of kidney dysfunction. Prevalences of high USG (≥1.030), low urinary pH (≤5.5) and high BUN/SCr ratio (>20) were used as indicators of dehydration.

Self-reported social and work history items, diseases and medications, and heat stress exposure variables were dichotomised. A category of high tobacco consumption was created with subjects in the upper quartile of ever smokers (≥3 pack-years) and a category of high alcohol consumption composed of subjects in the upper tertiles of lifetime alcohol consumption (≥80 000 g) or average weekly consumption (≥125 g). Total fluid intake was defined as drinking water plus sugary drinks (natural fruit refreshments, sodas, coffee, tea and electrolyte solution) and reported as litres of total liquids consumed the previous (work) day and for comparison also for a typical non-work day, with subcategories into water only and sugary drinks. Total fructose intake was estimated from all food and fluids consumed including chewed cane and stratified into fructose from food sources and added sugars. Fructose variables were categorised into quartiles. The cut-off for body mass index was set at ≥25 kg/m^2. Hypertension was defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg, or a self-reported medical history of hypertension. Diabetes was defined as serum glucose ≥125 mg/dL in the fasting serum sample or a self-reported medical history of diabetes. Use of nephrotoxic medications was recorded if taken at least three times per week for more than 3 months in the case of NSAIDs and other analgesics, or administered for at least a week in the case of nephrotoxic antibiotics, during the last year. Blood and urine biochemical parameters were explored as continuous variables or defined as normal versus abnormal using standard clinical cut-off values.

Differences between occupations were assessed with ANOVA and Kruskal-Wallis tests for normally and not normally distributed continuous variables, respectively, and Pearson χ^2 test for categorical variables or Fisher's exact test when the χ^2 test was not applicable. Post hoc tests were performed with Tukey's HSD test for continuous results and post-hoc χ^2 as described by Franke *et al*.[27] With occupation as the main proxy for heat stress, we assessed trends for sugarcane cutters > construction workers > farmers for prevalences of markers of kidney dysfunction and dehydration over the ordered occupational groups with the gamma statistic.

Differences in the distribution of risk factors between subjects with reduced and normal kidney function were explored for all occupations combined (n=194) and restricted to sugarcane cutters (n=86), with Whitney U-tests for continuous variables and χ^2 tests or Fisher's exact test for categorical variables. Exact p values are reported and p values ≤0.05 were considered statistically significant. Multivariate linear regression models were constructed for all workers and restricted to sugarcane cutters, with factors that were different between subjects with reduced and normal kidney function at p<0.10. Residuals from the regressions were checked to assess the fit of the models.

RESULTS

Potential risk factors for CKD/MeN among the three occupations

Socioeconomic and health-related CKD risk factors

Socioeconomic CKD risk indicators were unfavourable for all workers, but somewhat less for construction workers (table 1A). Farmers had the lowest income and sugarcane cutters were significantly less educated with an average of 4 years of elementary schooling. With

Table 1 Socioeconomic and health indicators relevant for chronic kidney disease/Mesoamerican nephropathy risk among workers in three occupations, municipalities of Chinandega and León, Nicaragua, 2013

	Sugarcane (N=86)	Construction (N=56)	Farming (N=52)	p Value* differences between groups
(A) Demographics, employment and social indicators				
Age (years)	25.6±5.5	27.3±6.0	25.2±5.1	0.11
Education (years)	3.9±3.0†	7.8±3.6	8.0±4.1	<0.001
Drinking water from well, %	84.9†	12.5	13.5	<0.001
Temporary contract, %	93.0†	75.0†	21.1†	<0.001
Without work ≥4 months/year, %	20.9	17.9	34.6†	0.089
No current social security, %	15.1	8.9	92.3†	<0.001
Monthly household income per person in family (25 córdobas=1 US$)	1808±1156†	2267±1124†	1343±1059†	<0.001
(B) Lifestyle, medical history and health indicators				
High tobacco consumption, %	10.5†	26.8	23.1	0.031
High alcohol consumption, %	18.6	28.6	32.7	0.145
Non-steroidal anti-inflammatory drugs ≥3 months, %	5.8	7.1	7.7	0.901
Nephrotoxic antibiotics, %	1.2	1.8	0.0	0.648
History of kidney stones, %	1.2	5.4	1.9	0.287
History of urinary tract infections, %	23.3‡	33.9	42.3‡	0.058
Not feeling in good health, %	10.5§	37.5§	17.5	<0.001
Body mass index ≥25 kg/m^2, %	17.4§	58.9§	40.4	<0.001
Blood pressure>140/90, %	5.8†	17.9	26.9	0.003
Heart rate (beats/min)	62±12†	73±14	72±13	<0.001
Blood glucose (mg/dL)	89±11	90±14	90±12	0.874
Triglycerides (mg/dL)	120±67†	168±108	177±124	<0.001
Cholesterol (mg/dL)	170±36§	188±41§	178±44	0.032
HDL cholesterol (mg/dL)	48±12†	42±10	38±8	<0.001
LDL cholesterol (mg/dL)	93±28	101±33	91±32	0.120
VLDL cholesterol (mg/dL)	24±13†	34±22	35±25	<0.001
Haematocrit (%)¶	46.8±5.9	48.5±4.8	50.8±4.0†	<0.001
Haemoglobin (g/dL)¶	13.4±1.6†	14.8±1.5	15.4±1.3	<0.001
Haemoglobin <13 g/dL, %¶	35.8†	8.9	3.8	<0.001
White cell counts/µL¶,**	7184±2048	7307±1656	7580±1882	0.503
% neutrophils¶,**	38.6±10.6	38.6±8.8	36.5±9.0	0.421
% lymphocytes¶,**	21.2±6.6†	18.5±4.9†	14.5±4.7†	<0.001
% other cells¶,**	40.2±10.0	43.0±8.2	49.1±10.6†	<0.001
Erythrocytes×10^6/µL¶	4.87±0.59†	5.27±0.47†	5.53±0.50†	<0.001
Platelets×10^3/µL¶	299.4±76.7	315.6±67.7	292.8±62.8	0.218

Values are mean±SD unless indicated otherwise.
*p Value for differences between groups: ANOVA for normally distributed continuous variables, Kruskal-Wallis for not normally distributed continuous variables, χ^2 test for categorical variables.
†Significantly different from the other two categories in post hoc tests.
‡Significant difference only between sugarcane cutters and farmers.
§Significant difference only between sugarcane cutters and construction workers.
¶5 missing data for sugarcane workers due to technical error.
**Exclusion of one farmer with outlier for white blood cell count (WBC count=17500/µL).
HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

regard to lifestyle and medical factors (table 1B), sugarcane cutters had lower prevalences of high tobacco and alcohol consumption. There were no major differences in the use of nephrotoxic drugs between the groups. None of the workers had been previously diagnosed with diabetes and only five had hyperglycaemia >125 mg/dL (2 sugarcane cutters, 2 construction workers and 1 farmer). Sugarcane cutters showed less obesity, better lipid profiles, lower heart rates and lower blood pressure but more anaemia (36% with haemoglobin <13 g/dL). There were no differences in total leucocyte count between occupations.

Occupational heat exposure, fluid and fructose intake and pesticides

On average, construction workers had an effective work time of 8 hours and farmers had the shortest with 5 hours, whereas sugarcane workers actively cut cane for 6.5 hours per day (table 2A). A higher proportion of sugarcane workers perceived a very rapid work pace and had to take rest breaks in the absence of shade; 83% received incentives for cutting more cane and almost half started harvesting within 12 hours of burning the cane. Sugarcane cutters more often reported weight loss related to the current job (over the last 2 months) and

Table 2 Occupational heat stress, fluid and fructose intake and pesticide exposure indicators among workers in three occupations, municipalities of Chinandega and León, Nicaragua, 2013

	Sugarcane (N=86)	Construction (N=56)	Farming (N=52)	p Value*
(A) Current occupational heat stress				
Effective work hours per day (work hours minus breaks)	6.5±1.2†	8.1±0.7†	5.3±2.0†	<0.001
Very rapid work pace, %	74.4†	53.6	40.4	<0.001
No shade during breaks, %	20.9‡	1.8‡	11.5	0.004
Lifting weights >50 lbs, %	18.6†	66.1	65.4	<0.001
Awkward work postures, %	58.1	76.8	69.2	0.063
Incentives to cut more cane, %	82.6	–	–	–
Hours post-burning at field entrance	11.7±6.2	–	–	–
Self-reported weight loss on the current job (last 2 months), %	77.9†	39.3	36.5	<0.001
Fainted at work, %	5.8	0	1.9	0.126
Dysuria ('chistata'), %	43.0	48.2	44.2	0.827
(B) Fluid and fructose intake				
Fluid intake previous day (workday)				
Total fluid (L)	6.2±4.1†	4.4±2.1	4.0±2.7	0.003
Water	4.4±3.9†	2.9±2.1	2.8±2.4	0.002
Sugary drinks without electrolyte hydration solution	1.8±1.8	1.5±0.9	1.2±0.8	0.208
Electrolyte solution (N=31)	1.2±1.1	–	–	–
Lowest quartile total fluid (≤2.5 L), %	18.6	19.6	40.4†	0.009
Highest quartile total fluid (≥7.0 L), %	40.7†	8.9	13.5	<0.001
(C) Fluid intake on typical non-work day				
Total fluid (L)	4.2±2.3	3.8±1.7	4.1±2.2	0.503
Water	3.0±2.0	2.2±1.3	2.7±2.0	0.053
Sugary drinks	1.2±1.1	1.6±1.1	1.4±1.9	0.117
(D) Fructose intake previous day (workday)				
Total fructose intake (g)	103.1±72.1†	80.1±46.1	70.9±36.8	0.008
From food sources	8.4±10.7†	15.9±16.6	17.4±16.7	<0.001
From added sugar	94.7±70.5†	64.2±38.1	53.2±30.7	<0.001
During work hours	58.6±44.7†	28.6±21.4	26.1±16.5	<0.001
Sugary drinks ('frescos', sodas, coffee)	22.5±15.7	28.6±21.4	26.1±16.3	0.108
Sugarcane chewing (N=53)	35.0±18.5	–	–	–
Electrolyte solution (N=31)	40.3±35.2	–	–	–
Outside (before and after) work hours	36.1±39.3	35.6±31.4	27.1±25.9	0.350
Highest quartile total fructose intake (>107 g), %	40.7†	19.6	15.7	0.002
(E) Work and pesticide use history				
Cumulative time on current job (months)	77±60	68±80	116±67†	0.001
Ever sugarcane work, %	100.0†	3.6	3.8	<0.001
Ever plantation (other than sugarcane), %	24.4	5.4†	21.2	0.012
Ever work in small-scale agricultural (%)	61.6†	25.0†	100.0†	<0.001
Ever construction work, %	5.8	100.0†	11.5	<0.001
Ever any pesticide use, %	46.5†	10.7†	71.2†	<0.001
Glyphosate, %	19.8†	0.0	3.8	<0.001
2,4-D, %	23.3†	0.0†	9.6†	<0.001
Paraquat, %	9.3	3.6	25.0†	0.002
Chlorpyrifos, %	0.0	0.0	23.1†	<0.001
Cypermethrin, %	18.6†	3.6†	42.6†	<0.001

Values are mean±SD unless indicated otherwise.
*p Value for differences between groups: ANOVA for normally distributed continuous variables, Kruskal-Wallis for not normally distributed continuous variables, χ^2 test for categorical variables.
†Significantly different from the other two categories in post hoc tests.
‡Significant difference only between sugarcane cutters and construction workers.

fainting on the job (6% compared with 2% of farmers and no construction workers). Dysuria ('chistata'), a common symptom in MeN affected areas thought to be related to dehydration,[15 24] was not different between the three groups.

With regard to fluid intake (table 2B), sugarcane cutters reported on average 6.2 L of total fluid intake the previous (work) day, 70% (4.4 L) as water and almost 30% (1.8 L) as sugary drinks. This was higher than for construction workers and farmers. Intake of

water and sugary beverages were not correlated (r_p=0.01). In contrast, there was no difference between the three groups for total fluid, water and sugary drinks intake on non-work days.

Fructose intake during the previous day was highest for sugarcane cutters and 41% of sugarcane cutters belonged to the category of highest quartile of consumption of total fructose (>107 g) (table 2B). Fructose intake from food was low among sugarcane cutters and most came from added sugars during work hours, specifically from sweetened beverages, electrolyte hydration solution (one-third of cutters) and cane chewing (about two-thirds). Fructose intake outside work hours was not different between the groups.

With regard to pesticide exposures (table 2C), farmers used pesticides most frequently (71%) compared with almost half of sugarcane cutters and only 11% of construction workers. Glyphosate and 2,4-D use was more common among sugarcane cutters whereas paraquat and the insecticides cypermethrin and chlorpyrifos were used more often by farmers. With the exception of cypermethrin, which had been used by almost half of the farmers, no specific pesticide exceeded 25% of users in any of the groups.

Status of kidney function and hydration by occupation

Kidney function biomarkers were more commonly abnormal among sugarcane cutters, with significant differences between the groups for prevalences of eGFR <80 mL/min/1.73 m^2 (16%, 9% and 2% in sugarcane cutters, construction workers and small-scale farmers, respectively; p for trend=0.003), high SCr (p for trend=0.02) and high BUN (p for trend=0.003) (table 3A). Likewise, proteinuria >30 mg/dL was approximately three times more prevalent in sugarcane workers than in the other groups (15% vs 5–6%, p for trend=0.08), whereas leucocyturia was observed in 22% of sugarcane workers but in only 0–2% of the other heat-exposed groups (p<0.001). Microhaematuria was also three times more prevalent in sugarcane workers, but this difference was not statistically significant (6% vs 2%, p for trend=0.19). High S-UA was more common among sugarcane cutters (17%) and construction workers (16%) than among farmers (6%).

Regarding markers of dehydration, the prevalence of concentrated urine (USG ≥1.030) was not statistically different between groups (table 3B). Low urinary pH occurred in 29% of sugarcane cutters versus 12% of construction workers and farmers (p=0.01) and sugarcane

Table 3 Biomarkers of kidney function and dehydration among workers in three occupations and trend over categories ordered by exposure to occupational heat stress (sugarcane > construction > farming), municipalities of Chinandega and León, Nicaragua, 2013

Variable	Sugarcane (N=86)	Construction (N=56)	Farming (N=52)	p Value: differences between groups*	p Value: trend†
(A) Indicators of kidney function					
BUN (mg/dL), mean±SD (range)	13.9±5.0‡ (6.0–28.4)	10.1±5.1 (4.1–30.0)	9.2±3.6 (4.0–22.0)	<0.001	
BUN >20 mg/dL (%)	15.1‡	5.4	1.9	0.017	0.003
Serum creatinine (SCr) (mg/dL), mean±SD (range)	0.84±0.39 (0.44–2.39)	1.00±1.16 (0.49–8.84)	0.78±0.22 (0.51–1.83)	0.393	
SCr >1.2 mg/dL, %	17.4¶	8.9	5.8¶	0.088	0.024
$eGFR_{CKD\text{-}EPI}$, mean±SD (range)	121±31 (34–160)	118±30 (7–161)	125±18 (49–158)	0.299	
$eGFR_{CKD\text{-}EPI}$ <80 mL/min/1.73 m^2, %	16.3¶	8.9	1.9¶	0.025	0.003
S-UA (mg/dL), mean±SD (range)	6.0±1.7 (3.0–12.7)	5.8±1.6 (3.6–11.0)	5.0±1.1‡ (2.9–8.1)	0.001	
S-UA >7.2 mg/dL, %	17.4	16.1	5.8	0.136	0.055
Proteinuria >30 mg/dL, %	14.7	5.4	6.1	0.128	0.081
Leucocytes in urine, %	22.1‡	0	1.9	<0.001	<0.001
Nitrites in urine, %	0	0	0	–	–
Blood in urine, %	5.8	1.8	1.9	0.339	0.186
(B) Indicators of dehydration					
Urinary specific gravity ≥1.030, %	15.3	28.6	20.4	0.161	0.255
Urinary pH ≤5.5, %	29.4‡	12.5	12.2	0.014	0.006
BUN/SCr ratio >20, %	25.6‡	0	3.8	<0.001	<0.001

*p Value for differences between groups: ANOVA for normally distributed continuous variables, Kruskal-Wallis for not normally distributed continuous variables, χ^2 test for categorical variables.
†Gamma statistic for trend over ordered categories.
‡Significantly different from the other two categories in post hoc tests.
¶Significant difference only between sugarcane cutters and construction workers.
BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; SCr, serum creatinine; S-UA, serum uric acid.

cutters more commonly had an elevated BUN/SCr ratio (26% vs 0 and 4% of construction workers and farmers, p<0.001). Trends over ordered categories were significant for urinary pH and BUN/SCr ratio. Although sugarcane cutters as a group had a lower prevalence of concentrated urine, within the group low fluid intake was strongly associated with concentrated urine (OR 3.5, p=0.06) and acidic urine (OR 8.7, p<0.001), which was not the case among construction workers and farmers (table 4).

Risk factors for reduced kidney function

In bivariate analyses of differences in kidney, urinary and metabolic biomarkers, work practices, hydration practices and lifestyle characteristics between subjects with reduced kidney function (eGFR <80 mL/min/1.73 m^2) and subjects with normal kidney function (eGFR ≥80 mL/min/1.73 m^2) (see online supplementary table S1), reduced kidney function was significantly associated with work as a sugarcane cutter, high intake of water, low intake of sugary beverages, increasing age, low haemoglobin and high tobacco consumption. In analyses restricted to sugarcane cutters the results were similar and, in addition, workers with reduced kidney function had cut cane for a considerably longer time than those with normal kidney function (cumulative time on the job: median 108 vs 60 months, p=0.06). Sugarcane cutters with reduced kidney function reported almost three times higher water intake and three times lower intake of sugary beverages than cutters with normal kidney function, with only one of the 14 reporting intake of the electrolyte solution. In addition, the cane cutters with reduced kidney function had a worse lipid profile than those with normal kidney function and more often had hypertension, but none had diabetes or hyperglycaemia and only one was overweight (see online supplementary table S1).

In backwards stepping multivariate linear regression analyses with inclusion of variables with p≤0.10 in the bivariate analyses (except haemoglobin due to missing data), age (β −1.3, 95% CI −1.8 to −0.8; p<0.001) and S-UA (β −10.4, 95% CI −12.2 to −8.5; p<0.001) were significantly associated with reduced kidney function among all workers, which was identical in models with total fluid intake and with intake of water and sugary beverages separately (table 5A). In the subset of sugarcane cutters, too many variables had a p value ≤0.10 in bivariate analyses (see online supplementary table S1) and therefore the regression was done in two steps. Hypertension, lipid profile tests and blood sugar were not associated with reduced kidney function in a model also including age and S-UA (data not shown) and were not further considered. In a model with water intake, intake of sugary drinks (without electrolyte solution) and intake yes/no of electrolyte solution, age, S-UA, high tobacco consumption and high alcohol consumption (table 5B), reduced kidney function was associated significantly with age and S-UA and non-significantly with the intake of electrolyte solution (β 8.1, 95% CI −1.2 to 17.5, p=0.09). Age and cumulative months on the job correlated (r_p 0.68, p<0.001), and substituting age with time cutting cane yielded similar results.

DISCUSSION

This study found evidence for more frequent heat stress, dehydration and kidney dysfunction among sugarcane

Table 4 Associations between low intake of fluids and markers of dehydration among sugarcane cutters (n=86) and non-cutters (construction workers and small-scale farmers) (n=108)

Lowest quartiles of fluid intake	USG ≥1.030* OR (95% CI) p Value†	pH ≤5.5*	BUN/SCr ratio >20*
Total fluids ≤2.5 L			
Sugarcane cutters (n=16)	3.5 (1.0 to 13) p=0.06	8.7 (2.6 to 29) p<0.001	1.2 (0.3 to 4.3) p=0.67
Construction workers and farmers (n=32)	1.4 (0.5 to 3.5) p=0.51	2.3 (0.7 to 7.5) p=0.17	–‡
Water ≤1.5 L			
Sugarcane cutters (n=16)	3.0 (0.7 to 12) p=0.14	2.9 (0.9 to 9.6) p=0.08	2.3 (0.7 to 7.3) p=0.17
Construction workers and farmers (n=32)	1.9 (0.7 to 4.9) p=0.18	1.7 (0.5 to 5.6) p=0.42	–‡
Sugary drinks ≤0.75 L			
Sugarcane cutters (n=28)	2.5 (0.7 to 9.2) p=0.16	2.5 (0.9 to 7.1) p=0.08	0.3 (0.2 to 1.1) p=0.06
Construction workers and farmers (n=21)	1.8 (0.6 to 5.2) p=0.28	0.7 (0.2; 3.6) p=0.69	–‡

*Markers of dehydration: high USG (≥1.030), acidic urine (urinary pH ≤5.5), high BUN/SCr ratio (>20).
†The ORs and 95% CIs for water and sugary drinks are adjusted for each other.
‡Not computed because only two non-cutters had BUN/SCr ratio >20.
BUN, blood urea nitrogen; SCr, serum creatinine; USG, urinary specific gravity.

Table 5 Multivariate linear regression models of estimated glomerular filtration rate ($eGFR_{CKD-EPI}$) among all workers (sugarcane cutters, construction workers and farmers) and restricted to sugarcane cutters

	β coefficient (95% CI)	Standardised β coefficient	p Value	Adjusted R^2
(A) All subjects (N=194)				
Step 1				
Water intake (L)	−0.7 (−1.7 to 0.3)	−0.08	0.15	0.47
Sugary beverages intake (L)	1.2 (−0.8 to 3.3)	0.06	0.24	
Sugarcane cutter ever	3.6 (−2.5 to 9.6)	0.07	0.25	
Age (years)	−1.2 (−1.7 to −0.6)	−0.24	<0.001	
Serum uric acid (mg/dL)	−10.0 (−12.0 to −8.1)	−0.57	<0.001	
High tobacco consumption	−4.5 (−11.6 to 2.7)	−0.07	0.22	
High alcohol consumption	1.2 (−5.6 to 8.1)	0.02	0.72	
Final step				
Age (years)	−1.3 (−1.8 to −0.8)	−0.27	<0.001	0.47
Serum uric acid (mg/dL)	−10.4 (−12.2 to −8.5)	−0.59	<0.001	
(B) Sugarcane cutters (N=86)				
Step 1				
Water intake (L)	−0.7 (−1.9 to 0.5)	−0.09	0.25	0.58
Sugary beverages intake (without electrolyte solution) (L)	1.2 (−3.7 to 6.0)	0.04	0.63	
Electrolyte solution (yes/no)	6.4 (−4.5 to 17.3)	0.10	0.24	
Age (years)	−1.7 (−2.5 to −0.8)	−0.29	<0.001	
Serum uric acid	−10.9 (−13.8: −8.1)	−0.59	<0.001	
High tobacco consumption	−10.1 (−22.5 to 2.3)	−0.12	0.11	
High alcohol consumption	−7.8 (−19.5 to 3.9)	−0.10	0.19	
Final step				
Age (years)	−1.9 (−2.7 to −1.1)	−0.34	<0.001	0.57
Serum uric acid (mg/dL)	−11.3 (−14.0 to −8.6)	−0.61	<0.001	
Electrolyte solution (yes/no)	8.1 (−1.2 to 17.5)	0.13	0.09	

cutters, as expected, and to a lesser degree also reduced kidney function among construction workers but not among small-scale farmers. Also, as expected, S-UA levels increased with decreasing eGFR.

Evidence of reduced kidney function

We used a cut-off of eGFR of 80 mL/min/1.73 m^2 to evaluate differences in renal function because only 11 workers had eGFR <60 due to their young age (all under age 40) and also because sugarcane workers were screened by employers before the start of the harvest 2 months earlier and workers with SCr >1.2 mg/dL were not hired and, thus, were not part of our study population. Despite this, approximately one-quarter of sugarcane cutters had evidence for either eGFR <80 mL/min/1.73 m^2, SCr >1.2 mg/dL or proteinuria ≥30 mg, and these findings were, respectively, eight-, three- and twofold more common than those observed in subsistence farmers and about twofold more common than in construction workers (table 3). However, although to a lesser degree than cane cutters, construction workers also had an unusually high prevalence of decreased kidney function, which is in accordance with a previous unpublished study in the same area.[25] In contrast, the single small-scale farmer with reduced kidney function had worked previously in sugarcane. Thus, our results show that not all agricultural workers are at increased risk for CKD, as is commonly stated, but rather workers in certain types of agriculture and other jobs in the heat such as work in the construction industry. The absence of reduced kidney function among subsistence farmers is consistent with a study in a MeN epidemic area in El Salvador, where subsistence farmers without a history of plantation work had a significantly lower prevalence of abnormal SCr than men who had worked on sugar or cotton plantations (15% vs 33%).[20] Reduced kidney function was accompanied by a higher frequency of anaemia among sugarcane cutters (36% vs 4–9% in the other groups). The prevalence of anaemia was higher than the prevalence of reduced kidney function and cannot be simply ascribed to the higher frequency of reduced renal function. Marked anaemia, defined as Hb <10 g/dL, was not observed in any of the groups.

Reduced kidney function was not associated with traditional risk factors for CKD. Notably, there were no cases of confirmed diabetes in the entire population. Importantly, sugarcane workers showed significantly worse renal function despite an overall lower frequency of abnormal lipid profile, hypertension and obesity compared with the other two groups (see table 1). Increasing age (>50 years) is a known risk factor for CKD, but in our study increasing age was associated with a decline in renal function despite the young age of the study participants. This is possibly related to an increased risk with continued job exposure over time, in particular among the sugarcane cutters. Thus, our study

suggests that most cases of reduced kidney function are related to MeN and not classic CKD.

Evidence for heat stress

There was evidence for a greater risk of heat stress among sugarcane cutters. Sugarcane cutters laboured at a faster pace, had less exposure to shade, reported more weight loss during the ongoing harvest and had more fainting episodes. While sugarcane cutters had greater heat stress exposure, they also drank more fluids during the course of the day, amounting to an average of 6.2 L per day (although this varied considerably, with approximately 20% drinking <2.5 L/day and 40% >7 L/day). However, the type of exertion and sweating that occurs with cane harvesting[11–13] could still result in a dramatic loss of fluids such that dehydration can occur despite high fluid consumption. Cade *et al*[28] found that college football players could lose as much as 8 quarts (about 7.6 L) of water in a 2-hour period, associated with loss of salt, decrease in blood glucose and a fall in blood pressure.

Potential mechanisms involved in inducing kidney damage

Daily heat stress and dehydration may cause repeated renal hypoperfusion episodes, and intermittent subclinical rhabdomyolysis associated with excessive exertion may also induce repeated acute kidney injury through the release of inflammatory mediators including oxidants, cytokines and uric acid which, over time, leads to CKD.[18] Experimental evidence has shown that repeated exposure to heat stress caused a reduction in renal function accompanied by histological evidence of tubulointerstitial damage.[19] Heat stress is known to raise S-UA levels, in part from subclinical rhabdomyolysis[29] but also from reduced renal blood flow.[30] In turn, hyperuricaemia is a well-known risk factor for CKD[31] and mediates both glomerular and tubulointerstitial disease in animals.[32–34] Interestingly, S-UA levels tended to be highest in both sugarcane workers and construction workers, with 16–17% of these individuals having hyperuricaemia compared with 6% of subsistence farmers. Furthermore, we found that the presence of hyperuricaemia was independently and strongly associated with declining renal function —that is, for each increase of 1 mg/dL S-UA there was an average decline of 10 mL/min in kidney filtration (see table 5). However, since reduced renal function can also result in increased uric acid levels due to impaired excretion, the causal role of uric acid in reduced kidney function cannot be determined.

Recently we hypothesised that renal injury could be occurring in sugarcane workers due to cyclical uricosuria with crystal formation.[20 21] According to this hypothesis, S-UA might rise as a consequence of subclinical rhabdomyolysis, followed by its crystallisation in the urine. One factor that increases the risk of urate crystal formation is acidic urine, which could result from the release of lactic acid associated with strenuous exercise and the effects of dehydration to reclaim sodium with hydrogen ion excretion. Urine pH was significantly lower in the sugarcane workers compared with the other groups (see table 3) and was strongly associated with low fluid intake on the previous (work) day in the subset of sugarcane workers (see table 4). This might reflect the effects of greater volume depletion (with aldosterone stimulation), lactic acid generation during the previous day, or other mechanisms.

Hydration and fructose

We had expected that low water intake or high sugary fluid intake would be associated with reduced renal function, based on studies in animals.[19 35] However, workers with eGFR <80 mL/min/1.73 m^2 drank more water and consumed fewer sugar-based drinks during the workday than subjects with normal kidney function (4.5 vs 2.2 L water, p=0.08; 0.6 vs 1.25 L sugary beverages, p=0.001) (see online supplementary table S1). This was particularly so among the sugarcane cutters with reduced kidney function who drank about 4 L more water and 1 L less sugary beverages. Excessive thirst from decreased concentration capacity of impaired kidneys may partially explain these counterintuitive findings, as well as the very high water requirements during the heavy labour of sugarcane cutting.[12 13] Although low fluid intake was clearly associated with concentrated urine among sugarcane cutters (USG ≥1.030: OR 3.5, p=0.06) (see table 4), cutters in the quartile with the highest fluid intake did not have a decreased risk of concentrated urine (OR 1.3, p=0.70) while high fluid intake among non-sugarcane workers appeared to be preventive (OR 0.10, p=0.06). Salvadorian cane cutters who consumed amounts of fluid comparable to the Nicaraguan cutters were found to have insufficient fluid intake under their work conditions.[11]

Sugary beverages that contain fructose are known to increase the risk of albuminuria[36] and can induce renal injury in laboratory animals.[35] However, fructose is also a component of sports drinks and fluid resuscitation packets containing glucose and electrolytes that might be beneficial to the volume and water depleted, such as by providing glucose that may prevent or treat any associated hypoglycaemia or by helping to maintain blood pressure due to the fructose component.[37 38] In our study, the intake of electrolyte solutions tended to be associated (p=0.09) with improved kidney function in multivariate analyses (see table 5). One study in Nicaragua found that, for each 100 mL electrolyte hydration packet consumed during the workday, the eGFR of cane cutters increased by 7 mL/min/1.73 m^2 over the course of one harvest season.[17] These issues need to be assessed with prospective studies that examine overall fluid balance by measuring fluid intake as well as losses during work, such as pre- and post-shift weight and serum and urine osmolarity.

Other risk factors for kidney disease

There was no association with NSAIDs or alcohol intake. A history of high tobacco consumption was more frequent among subjects with reduced kidney function (p=0.02) but lost significance in multivariate analyses. A history of pesticide exposure was more common among farmers, although exposure to herbicides was more common among sugarcane cutters, especially glyphosate and 2,4-D, both of special interest. However, analyses failed to identify pesticide exposures as an independent risk factor for reduced kidney function (see online supplementary table S1).

Study limitations

The main limitation of our study is its cross-sectional design. The kidney function parameters are based on single determinations in blood and urine without a chronicity criterion (presence during at least 3 months) for a proper clinical diagnosis of CKD.[39] Recently, attention has been drawn to the fact that single biomarker determinations and consequent categorisations into CKD stages based on a cut-off value, without consideration of age- and sex-specific criteria for GFR, are inadequate as the basis for population-based CKD prevalences because these practices can lead to overdiagnosis among the elderly and underdiagnosis in younger age groups, with large unexplained differences between nations.[40 41] However, the main purpose of our study is not a clinical diagnosis but to distinguish differences in kidney function parameters between three occupational groups of the same sex and same young age distribution, and comparisons therefore remain valid on the group level. In addition, in the same region, at the time of this study, we also followed a small group of heat-exposed sugarcane cutters and a group of control workers unexposed to heat over the harvest season. The cutters showed an important decline in kidney function,[42] which provides support for the cross-sectional findings, although no cohort data exist for construction workers or farmers.

Another limitation is that our heat exposure and hydration data were self-reported, but these data were collected through carefully designed questionnaires. Workers were asked to fast and did not consume any food before providing blood and spot urine samples between 05:30 and 06:00 hours (see Methods), but they did ingest water or other fluids during the evening, night and early morning. Nonetheless, we observed a lower U-pH and more frequent high BUN/SCr ratio among cane cutters and, to a lesser extent, among construction workers compared with subsistence farmers, which is an indication of incomplete recovery of adequate hydration status after the previous work day among the more heat stress-exposed workers.

Our sample size was based on a pre-study power calculation of 80% to detect CKD among 100 sugarcane cutters and100 non-cutters at α 0.05. Post hoc, we achieved a power of 0.68 for an increased risk of reduced eGFR among cutters versus non-cutters, but the post hoc power of the comparison between cutters and farmers was 80%. Therefore, our results seem sufficiently reliable, also considering the significant trends for indicators of heat stress, dehydration and kidney dysfunction in support of our main hypothesis of cane cutting>construction>farming. Finally, we did not have resources for examining biomarkers of early damage such as neutrophil gelatinase associated lipocalin (NGAL) and N-acetyl-β-D-glucosaminidase (NAG), which are important to include in future studies.

CONCLUSIONS

Compared with construction workers and, in particular, subsistence farmers from the same MeN epidemic region of Nicaragua, sugarcane cutters have higher heat stress, more dehydration and worse renal function despite the fact that other health indicators of the cutters were significantly better. Our study supports the need for improved work practices and even more hydration with adequate access to water for sugarcane cutters, as well as for workers in other hot occupations such as construction. The associations between intake of water and sugary drinks and kidney function as well as the role of hyperuricaemia need to be assessed in carefully designed follow-up studies.

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REFERENCES

1. Wesseling C, Crowe J, Hogstedt C, *et al*. Resolving the enigma of the Mesoamerican nephropathy—MeN—a research workshop summary. *Am J Kidney Dis* 2014;63:396–404.
2. Wijkström J, Leiva R, Elinder CG, *et al*. Clinical and pathological characterization of Mesoamerican nephropathy: a new kidney disease in Central America. *Am J Kidney Dis* 2013;62:908–18.
3. Ramirez-Rubio O, McClean MD, Amador JJ, *et al*. An epidemic of chronic kidney disease in Central America: an overview. *J Epidemiol Community Health* 2013;67:1–3.
4. Correa-Rotter R, Wesseling C, Johnson RJ. CKD of unknown origin in Central America: the case for a Mesoamerican nephropathy. *Am J Kidney Dis* 2014;63:506–20.
5. Torres C, Aragón A, González M, *et al*. Decreased kidney function of unknown cause in Nicaragua: a community-based survey. *Am J Kidney Dis* 2010;55:485–96.
6. Vela XF, Henríquez DO, Zelaya SM, *et al*. Chronic kidney disease and associated risk factors in two Salvadoran farming communities, 2012. *MEDICC Rev* 2014;16:55–60.
7. Orantes CM, Herrera R, Almaguer M, *et al*. Chronic kidney disease and associated risk factors in the Bajo Lempa region of El Salvador: Nefrolempa study, 2009. *MEDICC Rev* 2011;13:14–22.
8. Jayasinghe S. Chronic kidney disease of unknown etiology should be renamed chronic agrochemical nephropathy. *MEDICC Rev* 2014;16:72–4.
9. Wesseling C, Crowe J, Hogstedt C, *et al*. The epidemic of chronic kidney disease of unknown etiology in Mesoamerica: a call for interdisciplinary research and action. *Am J Public Health* 2013;103:1927–30.
10. Wesseling C, van Wendel de Joode B, Crowe J, *et al*. Mesoamerican nephropathy: geographical distribution and time trends of chronic kidney disease mortality between 1970 and 2012 in Costa Rica. *Occup Environ Med* 2015;72:714–21.
11. García-Trabanino R, Jarquín E, Wesseling C, *et al*. Heat stress, dehydration, and kidney function in sugarcane cutters in El Salvador—a cross-shift study of workers at risk of Mesoamerican nephropathy. *Environ Res* 2015;142:746–55.
12. Crowe J, Wesseling C, Román-Solano B, *et al*. Heat exposure in sugarcane harvesters in Costa Rica. *Am J Ind Med* 2013;56:1157–64.
13. Lucas RA, Bodin T, García-Trabanino R, *et al*. Heat stress and workload associated with sugarcane cutting—an excessively strenuous occupation! *Extrem Physiol Med* 2015;4(Suppl 1):A23.
14. Raines N, González M, Wyatt C, *et al*. Risk factors for reduced glomerular filtration rate in a Nicaraguan community affected by Mesoamerican nephropathy. *MEDICC Rev* 2014;16:16–22.
15. Ramirez-Rubio O, Brooks DR, Amador JJ, *et al*. Chronic kidney disease in Nicaragua: a qualitative analysis of semi-structured interviews with physicians and pharmacists. *BMC Public Health* 2013;13:350.
16. Jayasumana C, Fonseka S, Fernando A, *et al*. Phosphate fertilizer is a main source of arsenic in areas affected with chronic kidney disease of unknown etiology in Sri Lanka. *Springerplus* 2015;4:90.
17. Laws RL, Brooks DR, Amador JJ, *et al*. Changes in kidney function among Nicaraguan sugarcane workers. *Int J Occup Environ Health* 2015;21:241–50.
18. Paula Santos U, Zanetta DM, Terra-Filho M, *et al*. Burnt sugarcane harvesting is associated with acute renal dysfunction. *Kidney Int* 2015;87:792–9.
19. Roncal-Jimenez CA, Ishimoto T, Lanaspa MA, *et al*. Fructokinase activity mediates dehydration-induced renal injury. *Kidney Int* 2014;86:294–302.
20. Roncal-Jimenez C, Lanaspa MA, Jensen T, *et al*. Mechanisms by which dehydration may lead to chronic kidney disease. *Ann Nutr Metab* 2015;66(Suppl 3):P10–13.
21. Roncal-Jimenez C, García-Trabanino R, Barregard L, *et al*. Heat stress nephropathy from exercise-induced uric acid crystalluria: a perspective on Mesoamerican nephropathy. *Am J Kidney Dis* 2016;67:20–30.
22. McClean MA, Amador JJ, Laws R, *et al*. Biological sampling report: Investigating biomarkers of kidney injury and chronic kidney disease among workers in Western Nicaragua. Boston: University School of Public Health: Compliance Advisor Ombudman, 2012. http://www.cao-ombudsman.org/documents/Biological_Sampling_Report_April_2012.pdf (accessed 2 Jan 2016).
23. Peraza S, Wesseling C, Aragón A, *et al*. Decreased kidney function among agriculture workers in El Salvador. *Am J Kidney Dis* 2012;59:531–40.
24. Crowe J, Nilsson M, Kjellström T, *et al*. Heat-related symptoms in sugarcane harvesters. *Am J Ind Med* 2015;58:541–8.
25. Madero M, Arriaga JC, Jalal D, *et al*. The effect of two energy-restricted diets, a low-fructose diet versus a moderate natural fructose diet, on weight loss and metabolic syndrome parameters: a randomized controlled trial. *Metab Clin Exp* 2011;60:1551–9.
26. Agricultural Research Service. *National Nutrient Database for Standard Reference Release 27*. United States Department of Agriculture. http://ndb.nal.usda.gov/ndb/foods/show/6216?fg=&man=&lfacet=&count=&max=25&sort=&qlookup=cane&offset=&format=Full&new=&measureby (accessed 2 Jan 2016).
27. Franke TM, Ho T, Christie CA. The chi-square test: often used and more often misinterpreted. *Am J Evaluation* 2012;33:448–58.
28. Cade JR, Free HJ, De Quesada AM, *et al*. Changes in body fluid composition and volume during vigorous exercise by athletes. *J Sports Med Phys Fitness* 1971;11:172–8.
29. Vanholder R, Sever MS, Erek E, *et al*. Rhabdomyolysis. *J Am Soc Nephrol* 2000;11:1553–61.
30. Knochel JP, Dotin LN, Hamburger RJ. Heat stress, exercise, and muscle injury: effects on urate metabolism and renal function. *Ann Intern Med* 1974;81:321–8.
31. Johnson RJ, Nakagawa T, Jalal D, *et al*. Uric acid and chronic kidney disease: which is chasing which? *Nephrol Dial Transplant* 2013;28:2221–8.
32. Nakagawa T, Mazzali M, Kang DH, *et al*. Hyperuricemia causes glomerular hypertrophy in the rat. *Am J Nephrol* 2003;23:2–7.
33. Kang DH, Nakagawa T, Feng L, *et al*. A role for uric acid in the progression of renal disease. *J Am Soc Nephrol* 2002;13:2888–97.
34. Sánchez-Lozada LG, Tapia E, Santamaría J, *et al*. Mild hyperuricemia induces vasoconstriction and maintains glomerular hypertension in normal and remnant kidney rats. *Kidney Int* 2005;67:237–47.
35. Gersch MS, Mu W, Cirillo P, *et al*. Fructose, but not dextrose, accelerates the progression of chronic kidney disease. *Am J Physiol Renal Physiol* 2007;293:F1256–61.
36. Shoham DA, Durazo-Arvizu R, Kramer H, *et al*. Sugary soda consumption and albuminuria: results from The National Health and Nutrition Examination Survey, 1999–2004. *PLoS ONE* 2008;3:e3431.
37. Le MT, Frye RF, Rivard CJ, *et al*. Effects of high-fructose corn syrup and sucrose on the pharmacokinetics of fructose and acute metabolic and hemodynamic responses in healthy subjects. *Metab Clin Exp* 2012;61:641–51.
38. Brown CM, Dulloo AG, Yepuri G, *et al*. Fructose ingestion acutely elevates blood pressure in healthy young humans. *Am J Physiol Regul Integr Comp Physiol* 2008;294:R730–7.
39. Stevens PE, Levin A, Kidney Disease: Improving Global Outcomes Chronic Kidney Disease Guideline Development Work Group Members. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. *Ann Intern Med* 2013;158:825–30.
40. Benghanem Gharbi M, Elseviers M, Zamd M, *et al*. Chronic kidney disease, hypertension, diabetes, and obesity in the adult population of Morocco: how to avoid "over"- and "under"-diagnosis of CKD. *Kidney Int* 2016;89:1363–71.
41. De Broe ME, Gharbi MB, Elseviers M. Maremar, prevalence of chronic kidney disease, how to avoid over-diagnosis and under-diagnosis. *Nephrol Ther* 2016;12(Suppl 1):S57–63.
42. Wesseling C, Aragón A, González M, *et al*. Kidney function in sugarcane cutters in Nicaragua—a longitudinal study of workers at risk of Mesoamerican nephropathy. *Environ Res* 2016;147:125–32.

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LETTER

In reply to: "Should we consider renaming 'Mesoamerican Nephropathy' as Nephropathy of Unknown Cause in Agricultural Labourers (NUCAL)?"

We, from the Consortium on the Epidemic of Nephropathy in Central America and Mexico (CENCAM), read with interest Drs Subramanian and Javaid's letter,[1] regarding Mesoamerican Nephropathy (MeN), a name assigned to a type of chronic kidney disease (CKD) not related to classic risk factors and also referred to as CKD of unknown (CKDu) or non-traditional causes. MeN is highly prevalent in Central America and a major health problem.[2]

We agree with the authors that it is important to focus attention on the occupational component of this disease, and therefore on its preventable nature. Nevertheless, we consider it premature and inappropriate to rename MeN and other regional nephropathies as Nephropathy of Unknown Cause in Agricultural Labourers (NUCAL). First, this would imply that there is enough evidence to confirm that what is being described in Mesoamerica and other CKDu epidemics in developing nations (ie, Sri Lanka, India, Egypt) are manifestations of a single worldwide or multiregional disease. Moreover, the demographics reported in Mesoamerica and Sri Lanka are different. As an example, MeN is not limited to agricultural labourers only,[3 4] as the proposed name implies. In addition, histopathology in kidney biopsies from Mesoamerica and Sri Lanka, while having important similarities, suggest a predominantly tubulointerstitial type of disease with glomerulosclerosis, a non-specific histological pattern that does not provide at present significant information in relation to causality or prove a single entity.[5]

We believe that population-based, epidemiological, clinical and histopathology studies comparing between regions are urgently needed. While the exact pathogenesis of MeN is still uncertain and could be multifactorial, there is increasing evidence that strenuous occupational physical activity in hot environments without appropriate rest and rehydration may be playing an important role.[6]

Beyond any name discussion, we join the authors' plea for global awareness, and emphasise the urge for international action against this epidemic. There is limited access to proper renal replacement therapy in most of the affected nations. Progression to end stage renal disease is common among those suffering from MeN, and mortality is high.[3] Endemic pockets of CKDu have become a true humanitarian crisis. Collaboration globally among researchers, clinicians and other stakeholders will surely advance understanding. Funding and support for studies wherever CKDu is endemic is needed now, to clarify the disease's aetiology, in order to enable evidence-based prevention, and to combat its toll.[2]

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REFERENCES

1 Subramanian S, Javaid MM. 'Should we consider renaming 'Mesoamerican Nephropathy' as Nephropathy of Unknown Cause in Agricultural Labourers (NUCAL)?'. *Occup Environ Med* Published Online First: 1 July 2016. doi:10.1136/oemed-2016-103781
2 Wheelan E. The global epidemic of chronic kidney disease: a call for action. *Occup Environ Med* 2016;73:499–500.
3 García-Trabanino R, Hernández C, Rosa A, *et al*., On behalf of the Emergency Social fund for Health of Tierra Blanca. Incidence, mortality and prevalence of end-stage chronic renal disease in the Bajo Lempa region of El Salvador: a ten-year community registry. *Nefrologia* 2016;S0211–6995:30021–2.
4 Laux TS, Barnoya J, Cipriano E, *et al*. Prevalence of chronic kidney disease of non-traditional causes in patients on hemodialysis in southwest Guatemala. *Pan Am J Public Health* 2016;39:186–93.
5 Wijkström J, Leiva R, Elinder CG, *et al*. Clinical and pathological characterization of Mesoamerican nephropathy: a new kidney disease in Central America. *Am J Kidney Dis* 2013;62:908–18.
6 Roncal-Jimenez C, García-Trabanino R, Barregard L, *et al*. Heat stress nephropathy from exercise-induced uric acid crystalluria: a perspective on Mesoamerican nephropathy. *Am J Kidney Dis* 2016;67:20–30.

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Kidney function in sugarcane cutters in Nicaragua – A longitudinal study of workers at risk of Mesoamerican nephropathy

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ABSTRACT

Background: Chronic kidney disease is common among sugarcane workers in Central America. The main risk factor seems to be repeated high-intensity work in hot environments. Several cross-sectional studies have been performed but few longitudinal studies.
Objectives: The aim of the study was to examine whether kidney function changes over a few months of work during the harvest period.
Methods: A group of male sugarcane cutters in Nicaragua (N=29, aged 17–38 years) was examined with renal biomarkers before and after shift on the first day at the start of harvest, on the sixth day during acclimatization, and then in mid-harvest 9 weeks later. A reference group (N=25, mainly office workers) was examined with the same biomarkers at start of harvest, and then at end of harvest 5 months later.
Results: The pre-shift renal function decreased significantly during 9 weeks of work in the cane cutters. Mean serum creatinine increased (20%), mean estimated glomerular filtration rate decreased (9%, 10 mL/min), serum urea N (BUN) increased (41%), and mean urinary neutrophil gelatinase-associated lipocalin (NGAL) increased (four times). The cane cutters also developed cross-shift increases in these biomarkers, in particular serum creatinine and BUN, and in urinary uric acid. The longitudinal decrease in eGFR tended to be associated with the cross-shift increase in serum creatinine.
Conclusions: There was a remarkable decrease of glomerular kidney function, after only 9 weeks of harvest. The cross-shift increase in serum creatinine may be caused by dehydration (pre-renal dysfunction), and when repeated on a daily basis this may cause permanently reduced GFR.

1. Introduction

The recognition of Mesoamerican Nephropathy (MeN)-also labelled Chronic Kidney Disease of non-traditional origin (CKDnt) – as an epidemic in Central America, has led to the publication of a number of reports examining risk factors and causal hypotheses (Wesseling et al., 2014; Correa-Rotter et al., 2014). Experimental studies have also been performed (Roncal Jimenez et al., 2014).

To date the findings have shown that the epidemic primarily affects males working in heavy manual labor in hot environments, mainly living in the coastal lowlands. There is generally no history of diabetes or hypertension and no substantial proteinuria. Kidney

Abbreviations: BMI, Body mass index; CKD, Chronic kidney disease; eGFR, estimated glomerular filtration rate; KIM-1, Kidney injury molecule 1; MeN, Mesoamerican nephropathy; NGAL, neutrophil gelatinase-associated lipocalin; Hsp72, Heat shock protein 72 kD; NSAIDS, Non-steroid anti-inflammatory drugs; RAAS, renin-angiotensin-aldosterone system

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biopsies have shown a tubulointerstitial pattern with tubular atrophy and interstitial fibrosis, but also global glomerulosclerosis, often with an ischemic component (Wijkstrom et al., 2013; López-Marín et al., 2014).

The main hypothesis to account for the disease is heat stress with repetitive episodes of dehydration (Peraza et al., 2012; Brooks et al., 2012; Wesseling et al., 2014; Correa-Rotter et al., 2014; García-Trabanino et al., 2015). Suggested pathophysiologic mechanisms driven by strenuous work and heat stress include subclinical rhabdomyolysis (Paula Santos et al., 2015), effects of hyperuricemia and hyperuricosuria (Knochel et al., 1974; Johnson, 2015; Roncal-Jimenez et al., 2015, 2016), hyperosmolality-induced activation of the aldose reductase-fructokinase pathway in the kidney, and vasopressin effects (Roncal Jimenez et al., 2014; Roncal-Jimenez et al., 2015, 2016). It has also been proposed that the disease is multifactorial, and could include additional factors such as self-medication with nonsteroidal anti-inflammatory drugs, exposure to heavy metals or pesticides/agrochemicals, infections, or genetic factors (Correa-Rotter et al., 2014; Herrera et al., 2014; Laws et al., 2015a, 2015b; Ramírez-Rubio et al., 2015; Wesseling et al., 2014).

Most of the epidemiological studies are cross-sectional population based surveys. Two studies have examined cross-shift changes in biomarkers of hydration and kidney function, over a workday, in Brazil, (Paula Santos et al., 2015), and El Salvador (García-Trabanino et al., 2015), and two studies have performed a follow-up of kidney function among sugarcane cutters over the course of a harvest season, in Brazil (Paula Santos et al., 2015) and in Nicaragua (Laws et al., 2015a, 2015b). While the two studies on cross-shift changes both show a decrease in renal function over a cane cutting shift, the two longitudinal studies were not in agreement, and the question of whether the pre-shift glomerular function changes over a harvest period of several months still remains unclear. The aim of the present study was to assess longitudinal changes of kidney function over a harvest period in sugarcane cutters as well as in a reference group. We examined pre- and post-shift kidney function in sugarcane cutters at the start of harvest, on day 1 and day 6 to assess acclimatization effects, and at mid-harvest two months later. A reference group of non-cane cutters was examined at start and end of the harvest.

2. Methods

2.1. Setting and study design

The study was conducted in 2012–2013 in a convenience sample of 29 sugarcane cutters from León and Chinandega municipalities in the northern Nicaraguan Pacific region, and a reference group (N=25, mainly office workers) from the same area. The sugarcane cutters were examined "pre-shift" in the morning between 3 and 5 am and "post-shift" between 4 and 7 pm at their homes on the first day of the harvest in November 2012. The pre-shift examination on the first day (called Cut1) was considered to be the baseline. The examinations (pre- and post-shift) were repeated after 5 days of work (Cut2), and then 9 weeks later in January 2013 (in the mid-harvest period; Cut3). The first ("pre-shift" 7–9 am and "post-shift" 4–6 pm) examination of the reference group was at their work places in November 2012 (Ref1), while the repeated examination was performed at the end of the harvest season in May 2013 (Ref2), again at their workplaces. Originally, a fourth examination of the cane cutters was planned for the end of the harvest in May 2013, together with the reference group, but due to removal of participating workers from their jobs in February 2013, this could not be realized, apart from a small number (n=7) of post-shift urine samples.

2.2. Participants

Community leaders of six villages in the municipalities of León and Chinandega provided lists of men who planned to work as sugarcane cutters. Information meetings were held with these workers in their communities. An invitation to provide blood and urine for a screening test was made to those who were confirmed to be enrolled as sugarcane cutters at plantations of the mill in the study area. The aim was to recruit young healthy men. In total 92 sugarcane cutters <40 years of age participated in the screening test (blood tests for glucose, creatinine, uric acid, lipids, cell count, as well as a urine test with dip-stick and examination of sediment). These analyses were performed in the laboratory of the Medical School of UNAN-Léon. In 45 of them serum creatinine was ≥ 1.1 mg/dL, the strict pre-set exclusion criterion. Another 15 had abnormal results in at least one of the other tests, or a history of diabetes or hypertension. Thus only 32 workers met the inclusion criteria (<40 years of age, serum creatinine ≤ 1.0 mg/dL and all other lab tests within the reference values; in addition no known diabetes, hypertension or kidney disease). Three men decided not to participate, leaving 29 subjects for the study.

A reference group without known diabetes, hypertension or kidney disease was recruited at the town halls near the sugarcane plantations. The group included mostly office workers, but also five persons with a predominantly outdoor job, albeit without major physical effort.

All participants signed a written informed consent to participate in the study, in accordance with the Declaration of Helsinki. The study was approved by the Ethical Review Board of UNAN-León, Nicaragua, and the Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán, Mexico.

The work conditions were similar to those described previously for sugarcane cutting in this region (Crowe et al., 2015; García-Trabanino et al., 2015).

2.3. Medical examinations

Blood pressure was measured by a technician with a calibrated digital sphygmomanometer (Omron BP710N, Omron Healthcare Inc., Bannockburn, USA) with the participant seated after resting for at least 10 min. Body weight was measured with a calibrated Seca 803 digital flat mobile scale (Seca, Birmingham, UK) with minimal clothing and height with a foldable stadiometer (Seca, Birmingham, UK). Certified technicians collected blood samples in three vacuum tubes (Becton Dickinson & Co., USA), one tube with anticoagulant for blood cell count and two tubes with clot activator and gel for serum separation. All samples were placed on ice and transported immediately to the laboratory at the Research Center on Health, Work and Environment (CISTA) at UNAN-León, where they were centrifuged at 3500 RPM for 10 min at room temperature and the serum was separated into four labeled cryovials and stored at -80 °C.

Each participant delivered a spot urine sample (50 cc) in a sterile polypropylene container (Becton Dickinson & Co., USA), which was aliquoted into vacuum tubes, one with and two without preservative immediately at the participant's home, placed in an icebox (4 °C) and then transported to the laboratory at the Research Center on Health, Work and Environment (CISTA) at UNAN-León, where aliquots were frozen at -80 °C. Serum and urine aliquots were later sent to the Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubiran, Mexico (about seven months after collection), and urine aliquots to the University of Colorado Denver (within a month).

Baseline data were recorded by trained interviewers using a questionnaire recording data on age, education, smoking, alcohol, and some other background factors, as well as health (medically

diagnosed diseases and nephrotoxic medications), and work history.

2.4. Biochemical analyses

Sodium, potassium, calcium, uric acid, urea nitrogen ("BUN"), phosphate, and creatinine in serum were measured in Mexico City with an autoanalyzer (UniCel DxC 600, Beckman Coultier). Creatinine was calibrated against creatinine determined by isotope dilution mass spectrometry. Urine neutrophil gelatinase-associated lipocalin (uNGAL) and Kidney Injury Molecule 1 (uKim-1) levels were analyzed using commercially available enzyme-linked immune absorbent assay (ELISA) kits; uNGAL from BioPorto Diagnostics and uKim-1 from BioAssay Works. All procedures were performed according to manufacturers' instructions. For urinary heat shock protein 72 kD (Hsp72) detection by Western blot, 10 μL of each urine sample was loaded and resolved by 8.5% SDS-PAGE electrophoresis and electroblotted, as previously described (Barrera-Chimal et al., 2011). Membranes were then blocked with 5% blotting-grade non-fat dry milk and incubated in 0.1% blotting-grade non-fat dry milk with monoclonal Hsp72 antibody, 1:5000 (ENZO Life Science). Then, the detection of Hsp72 in urine was performed with goat anti-mouse antibody (1:5000 dilution) overnight at 4 °C (Santa Cruz Biotechnology). Proteins were detected with an enhanced chemiluminescence kit (Immobilon TM Western Chemiluminescent HRP substrate, Millipore) and autoradiography, following the manufacturer's recommendations. All Western blot analyses were performed within the linear range of protein loads and antibody use. The bands were scanned for densitometric analysis (E3 Bioctem Imaging System UVP, Upland CA) and densitometry was performed using Vision Works Software UVP.

Dipstick analyses of urine were also performed in connection with urine sampling using a Bayer Clinitek 50 Urine Chemistry Analyzer with Multistix 10SG reagent strips (Siemens Diagnostics, United States) for semi-quantitative measurements of proteinuria (at levels of ≥ 30 to < 300 mg/dL and ≥ 300 mg/dL, glucosuria (+ at ≥ 100 mg/dL), urinary specific gravity, pH, blood, nitrite, leukocytes, bilirubin, ketones and urobilinogen.

Studies performed at the University of Colorado included measurement of urine creatinine (Vet Ace analyzer), urine osmolarity (with Advance Micro Osmometer Model 3300) and urine pH (using a pH meter). Urinary fructose was measured using the EnzyChrom Fructose Assay Kit (BioAssay Systems, Hayward, CA). Urine uric acid was measured using the QuantiChrom TM Uric Acid kit assay (BioAssay systems) and included measurements of soluble uric acid and the uric acid in the pellet (the latter following correction of the pH to 7).

To normalize for differences in urinary flow rate, each urinary biomarker was adjusted for the urinary creatinine concentration. Estimated glomerular filtration rate (eGFR) per 1.73 m^2 of body surface area was calculated using the EPI-CKD formula based on serum creatinine (Levey et al., 2009).

2.5. Data analyses

Several variables were not normally distributed. Differences between groups were tested with Wilcoxon rank sum test or Fisher's exact test (for categorical variables). Differences between pre- and post-shift results were tested by Wilcoxon's signed rank test. Associations between variables were evaluated by the Pearson correlation coefficient (r_p).

For the key renal biomarker differences in sugarcane cutters between baseline (Cut1) and follow-up 9 weeks later (Cut3), as well as differences in referents between baseline (Ref1) and follow-up 5 months later (Ref2), were assessed by a mixed effects model. Skewed variables were log-transformed. In these models, with separate covariance matrices for cutters and referents, subject was a random factor and group (cutters and referents), day (first and last), and time (pre- and post-shift) were fixed effects. The model included the fixed effects and a three-way interaction term in order to assess the effect of group and day separately for pre- and post-shift results.

All analyses were repeated after exclusion of one sugarcane cutter who had a low eGFR already at baseline (sensitivity analysis). P-values < 0.05 were considered statistically significant. Data analyses were performed using SAS 9.4.

3. Results

Characteristics of the 29 sugarcane cutters and the 25 referents are shown in Table 1. The mean age of the cane cutters was 25 years. The referents were somewhat older, had slightly higher BMI, smoked slightly less, and used alcohol more often. In the interviews, one participant in each group reported hypertension, but both of them had normal blood pressure at examination and they did not take antihypertensive medications. Six cutters reported ever use of NSAIDS > 3 months versus one referent (P=0.11). In spite of pre-screening, one sugarcane cutter had a low eGFR at baseline, as determined post data collection at the laboratory in Mexico City.

3.1. Cross-shift changes

For the sugarcane cutters, body weight, heart rate, blood pressure, and results for serum and urine biomarkers over a workday at start of harvest (first and sixth work-day), and 9 weeks later are shown in Table 2A. There were several cross-shift changes. Body weight decreased somewhat on the first day, but not on the other days. Serum creatinine and serum urea N increased over

Table 1
Characteristics of the study population of sugarcane cutters and the reference group in Nicaragua, all men, at start of harvest. Mean, median (range) are shown for continuous variables, % for smoking habits, and numbers (N) for medical conditions.

	Cutters (N=29)	Reference group (N=25)	P-value
Age, years	25, 24 (17 – 38)	30, 31 (19 – 38)	< 0.001
Body weight, pre-shift	64, 60 (50 – 89)	75, 74 (52 – 103)	< 0.001
Height, cm	165, 166 (152 – 173)	169, 169 (159 – 178)	0.002
BMI, mean	24, 23 (18 – 31)	26, 27 (18 – 34)	0.01
Current smokers, %[a]	45	28	0.26
Ex-smokers, %	10	16	0.69
Current use of alcohol, %	41	72	0.03
Years of schooling	3, 3 (0 – 10)	13, 16 (0 – 16)	< 0.001
Hypertension, N[b]	1	1	
Diabetes, N[b]	0	0	
Nephrolithiasis, N[b]	1	0	
NSAIDs, N[c]	6	1	
Nephrotoxic antibiotics, N[d]	0	1	
S-creatinine (mg/dL), pre-shift[e]	0.96, 0.85 (0.65 – 2.4)	0.83, 0.81 (0.45 – 1.31)	0.07
eGFR mL/min/1.73 m^2[f]	111, 118 (37 – 141)	116, 120 (75 – 151)	0.49

[a] Mean number of cigarettes smoked per day was 7 in cane cutters and 4 in referents.
[b] Ever suffered (self-reported).
[c] Used for > 3 months.
[d] Gentamycin (at least one week in the past year).
[e] Workers were pre-screened for S-creatinine before harvest, and none had S-creatinine > 1.0 mg/dL by that time.
[f] One cutter had reduced eGFR (< 60 mL min/1.73 m^2).

Table 2A
Body weight, pulse rate, blood pressure, and results for serum and urine biomarkers over a work-day at start of harvest (first and 6th day) and 9 weeks later in 29 sugarcane cutters. Median (10–90-percentiles) or number (N) is given. P-values (Wilcoxon's signed rank test) for cross-shift changes are only presented if ≤0.05.

Day	Cut1 Nov 2012 first work-day N=29	P-value cross-shift change N=29	Cut2 Nov 2012 sixth work-day N=28	P-value cross-shift change N=26	Cut3 Jan 2013 N=28	P-value cross-shift change N=25
Body weight Pre (kg)	59.5 (57 – 80)		59.5 (57 – 80)		58.4 (55 – 72)	
Body weight change	−0.4 (−1.4 – 0.7)	0.02	0.2 (−1.0 – 1.3)		0.3 (−0.8 – 1.3)	
Pulse rate Pre (per min)	63 (50–84)		61 (50 – 74)		58 (45 – 68)	
Pulse rate change	10 (−15 – 27)	0.005	19 (2 – 30)	<0.001	11 (2 – 25)	<0.001
Systolic BP Pre (mm Hg)	130 (117 – 134)		122 (106 – 138)		118 (103 – 127)	
Systolic BP change	−7 (−23 – 21)	0.05	1 (−18 – 19)		2 (−11 – 10)	
Diastolic BP Pre (mm Hg)	83 (73 – 92)		81 (61 – 95)		80 (64 – 86)	
Diastolic BP change	−2 (−19 – 8)	0.04	−1 (−13 – 10)		−3 (−12 – 3)	0.01
Serum biomarkers						
S-creatinine Pre (mg/dL)	0.85 (0.67 – 1.2)		0.94 (0.67 – 1.3)		0.95 (0.77 – 1.6)	
S-creatinine change	0.12 (−0.04 – 0.21)	<0.001	0.06 (−0.01 – 0.15)	<0.001	0.08 (-0.12 – 0.35)	0.02
S-urea N Pre (mg/dL)	10.4 (7.5 – 17)		10.5 (6.7 – 16)		15.9 (8.9 – 20)	
S-urea N change	1.4 (−1.4 – 4.3)	0.002	0.9 (−1.6 – 3.7)	0.01	1.2 (−3.2 -3.5)	
S-uric acid Pre (mg/dL)	5.7 (4.3 – 7.5)		5.2 (3.6 – 7.1)		5.5 (4.5 – 7.2)	
S-uric acid change	−0.1 (−0.7 – 0.5)		0.2 (−0.2 – 0.7)	0.003	0.2 (−0.7 – 1.0)	
S-glucose Pre (mg/dL)	70 (60 – 86)		77 (65 – 84)		83 (76 – 93)	
S-Na Pre (mmol/L)	141 (138 – 144)		140 (138 – 142)		140 (138 – 142)	
S-Na change	0.1 (−2.2 – 2.5)		0.2 (−1.5 – 1.7)		−0.5 (−2.1 – 2.1)	
S-K Pre (mmol/L)	4.3 (3.6 – 5.2)		4.3 (3.6 – 4.7)		4.0 (3.4 – 4.8)	
S-K change	−0.5 (−1.2 – 0.3)		−0.1 (−0.5 – 0.5)		−0.2 (−0.9 – 0.5)	
S-Ca (total) Pre (mg/dL)	9.6 (9.1 – 10.2)		9.3 (8.7 – 9.8)		9.5 (9.0 – 10.1)	
S-Ca (total) change	−0.10 (−0.5 – 0.6)		0.15 (−0.3 – 0.5)		0.05 (−1.2 – 0.5)	
S-phosphate Pre (mg/dL)	4.6 (3.7 – 5.1)		4.4 (3.5 – 5.2)		4.2 (3.4 – 4.9)	
S-phosphate change	−0.2 (−0.9 – 0.6)		0 (−0.8 – 0.6)		−0.4 (−1.1 – 0.5)	0.005
Urine biomarkers						
pH Pre	6 (5.5 – 7)		6 (5.5 – 7)		6 (5.5 – 7)	
pH change	0.5 (−0.5 – 0.5)	0.02	0 (−0.5 – 1)		0 (−0.5 – 1)	
NGAL Pre (μg/gCr)	9.3 (2 – 48)		18 (4 – 89)		17 (6 – 285)	
NGAL change	1.4 (−31 – 29)		2.8 (−30 – 146)		6.3 (−18 – 118)	
KIM-1 (ng/gCr) Pre	4.7 (0.5 – 35)		11 (0.8 – 98)		4.6 (0.6 – 50)	
KIM-1 change	−0.2 (−17 – 34)		0.8 (−38 – 22)		2.8 (−3 – 23)	
Hsp Pre (N > LOD)	1		3		0	
Hsp Post (N > LOD)	7		3		3	
Creatinine Pre(g/L)	0.86 (0.3 – 2.0)		0.51 (0.3 – 1.7)		0.80 (0.3 – 1.5)	
Creatinine change	0.04 (−1 – 1.3)		0.12 (−1 – 1.4)		−0.01 (−0.06 – 0.03)	
Osmolality Pre	652 (356 – 923)		471 (332 – 836)		525 (350 – 877)	
Osmolality change	50 (−179 – 277)		46 (−375 – 393)		−1 (282 – 125)	
U-fructose Pre (μmol/gCr)	129 (64 – 458)		175 (80 – 312)		343 (119 – 686)	
U-fructose change	15 (−201 – 312)		103 (−90 – 513)	<0.001	114 (−157 – 583)	0.02
U-uric acid Pre (mg/gCr)	442 (304 – 653)		474 (332 – 810)		498 (310 – 1337)	
U-uric acid change (mg/gCr)	74 (−127 – 256)	0.02	104 (−49 – 263)	0.01	25 (−80 – 536)	

shift on all three sampling days. This was the case also if excluding the cutter with a low eGFR at baseline. The cross-shift changes of serum creatinine and serum urea N tended to be more marked in the six workers reporting use of NSAIDS (P=0.053 for cross-shift change of serum urea N at Cut1). The cross-shift changes in the referent group were less pronounced (Table 2B). Uric acid in urine increased over shift in the sugarcane workers, but not in the reference group. There was also a tendency (P=0.052) towards a cross-shift change in number of cutters with Hsp72 above the detection limit at Cut1.

3.2. *Longitudinal changes*

Table 2 also shows changes over time for all individuals. Over the nine-week period (first half of the harvest) the sugarcane workers decreased in body weight, heart rate and blood pressure pre-shift (Table 2A). In the reference group, re-examined after 5 months, body weight increased substantially, heart rate was unchanged, while blood pressure decreased (Table 2B). For the evaluation of longitudinal changes in key kidney function biomarkers we restricted the analysis to individuals who took part in examinations both at baseline and at end of follow-up (Table 3). Serum creatinine increased substantially in the sugarcane cutters over the nine-week period, pre-shift means from 0.98 mg/dL to 1.18 mg/dL, and serum urea N even more. Urinary NGAL also increased substantially, as did urinary uric acid, although mainly post-shift. Pre and post-shift serum phosphate and pre-shift serum potassium decreased among cane cutters. Although there were some changes in the reference group, the signs of deteriorated renal function were more pronounced in the sugarcane cutters. The mean estimated GFR decreased by 10 mL/min after only 9 weeks (reflecting the increase in serum creatinine), and in addition to one sugarcane cutter with reduced (< 60 mL/min) eGFR at start of harvest, two more cutters had reduced eGFR after nine weeks (Cut3). Some changes occurred already in the first week (see Table 2A): blood pressure decreased, urinary NGAL and KIM-1 increased, and there was a non-significant tendency towards an increase in serum creatinine. The sensitivity analysis excluding a cutter with low baseline eGFR showed that all statistically significant longitudinal changes in kidney function shown in Table 3 remained significant (data not shown).

There was a tendency towards an association between the cross-shift change of serum creatinine in the first week and the longitudinal change of eGFR over 9 weeks ($r_p = -0.40$, P=0.06 for association between cross-shift change of serum creatinine at Cut1 and long-term change of eGFR over 9 weeks, and $r_p = -0.42$,

Table 2B
Body weight, pulse rate, and blood pressure over a work-day and 5 months later in a reference group of 25 individuals. Median (10–90-percentiles) or number (N) is given. P-values (Wilcoxon's signed rank test) for cross-shift changes are only presented if ≤0.05.

Day	Ref1 Nov 2012 N=25	P-value cross-shift change N=25	Ref2 May 2013 N=25	P-value cross-shift change N=25
Body weight Pre (kg)	74 (56 – 89)		80 (57 – 90)	
Body weight change	0.0 (0 – 1.5)		1.6 (−2 – 4.5)	0.002
Pulse rate Pre (per min)	76 (63 – 85)		77 (65 – 82)	
Pulse rate change	4 (−4 – 15)	0.004	4 (−8 – 19)	
Systolic BP Pre (mm Hg)	119 (97 – 135)		111 (99 – 131)	
Systolic BP change	3 (−7 – 15)		2 (−8 – 17)	
Diastolic BP Pre (mm Hg)	81 (64 – 90)		72 (53 – 83)	
Diastolic BP change	−3 (−15 – 12)		3 (−6 - 18)	
Serum biomarkers				
S-creatinine Pre (mg/dL)	0.81 (0.66 – 1.1)		0.85 (0.71 – 1.1)	
S-creatinine change	0.08 (−0.02 – 0.29)	<0.001	0.04 (−0.08 – 0.20)	
S-urea N Pre (mg/dL)	9.6 (7.2 – 14)		9.8 (6.3 – 13)	
S-urea N change	1.2 (−2.1 – 5.7)	0.02	1.0 (−0.9 – 3.7)	0.002
S-uric acid Pre (mg/dL)	5.5 (4.0 – 8.1)		5.7 (4.1 – 7.4)	
S-uric acid change	0.0 (−0.7 – 0.7)		−0.3 (−0.6 – 0.7)	
S-glucose Pre (mg/dL)	81 (68 – 95)			
S-Na Pre (mmol/L)	140 (137 – 143)		140 (138 – 142)	
S-Na change	0.8 (−2 – 3)		0.8 (−2 – 4)	
S-K Pre (mmol/L)	4.4 (3.8 – 5.0)		4.2 (3.8 – 4.7)	
S-K change	−0.3 (−1.0 – 0.3)		0.1 (−0.4 – 0.4)	
S-Ca (total) Pre (mg/dL)	9.1 (8.7 – 9.6)		9.2 (8.7 – 9.5)	
S-Ca (total) change	0.1 (−0.4 – 0.7)		0.0 (−0.3 – 0.4)	
S-phosphate Pre (mg/dL)	3.5 (2.9 – 4.2)		3.5 (2.6 – 3.8)	
S-phosphate change	0.7 (−0.7 – 1.4)		0.6 (0.0 – 1.6)	
Urine biomarkers				
pH Pre	6 (5 – 7)		6.5 (5.5 – 7.5)	
pH change	0 (−1 – 1)		0 (−0.5 – 0.5)	
NGAL Pre (μg/gCr)	6.4 (0.9 – 22)		8.0 (2.0 – 46)	
NGAL change (μg/gCr)	0.1 (−13 – 14)		−0.3 (−23 – 27)	
KIM-1 Pre (ng/gCr)	3.1 (0.96 – 11)		3.5 (0.6 – 54)	
KIM-1 change	−0.6 (−3.6 – 12)		−0.2 (−12 – 22)	
Hsp corr Pre (N > LOD)	4		4	
Hsp corr Post (N > LOD)	4		3	
Creatinine Pre (g/L)	1.5 (0.4 – 2.0)		1.8 (0.5 – 3.8)	
Creatinine change	0.37 (−0.3 – 1.9)	0.01		0.01 (−0.9 – 1.5)
Osmolality Pre	837 (356 – 1023)		731 (220 – 1060)	
Osmolality change	90 (−272 – 320)		77 (−394 – 616)	
U-fructose Pre (μmol/gCr)	80 (63 – 191)		164 (69 – 1030)	
U-fructose change	116 (2 – 295)	<0.001		131 (−617 – 550)
U-uric acid total Pre (mg/gCr)	363 (171 – 549)		429 (299 – 667)	
U-uric acid change (mg/gCr)	−6 (−167 – 150)		6 (−121 – 186)	

P=0.06 for association between the mean of the cross-shift changes of serum creatinine at Cut1 and Cut2, and change of eGFR over 9 weeks).

3.3. Differences between cane cutters and referents

While there were no significant group differences in most indicators of renal function at baseline (comparison of Cut1 and Ref1 in Table 3), at follow-up cane cutters had significantly higher serum creatinine (pre- and post-shift), serum urea N (pre- and post-shift), and urinary NGAL (pre- and post-shift), and lower eGFR than referents (comparison of Cut3 and Ref2 in Table 3). Post-shift urinary uric acid was higher in cane cutters both the first day and at follow-up. Cane cutters and referents had similar pre-shift levels of serum potassium but at end of follow-up the post-shift potassium levels were significantly lower in cane cutters than in referents. Serum phosphate showed a different pattern. Pre-shift phosphate was higher in cane cutters than in referents, while post-shift levels at follow-up were not significantly different (Table 3). The statistically significant group differences remained after exclusion of a cutter with low baseline eGFR with one exception; the P-value for eGFR comparing Cut3 with Ref2 changed from 0.03 to 0.06.

3.4. Additional analyses

As mentioned in the Methods section, we only had seven post-shift urine samples from sugarcane cutters at the end of harvest ("Cut4" in May 2013). However, noteworthy is that those samples showed very high urinary uric acid values, with total urinary uric acid levels varying from 786 to 2413 mg/g creatinine (unadjusted uric acid 82–204 mg/dL), with a median of 1664 mg/g creatinine (unadjusted 134 mg/dL). All of these subjects were found to have urate crystals (dihydrate) in their urine. Interestingly, the days of collection represented some of the hottest days of the year for this region.

4. Discussion

In the present study a group of sugarcane cutters was examined on the first day at start of harvest, after 6 days, and again after 9 weeks. The most remarkable finding was a clear and significant decrease in pre-shift renal function after 9 weeks of work, with a

Table 3
Longitudinal changes in pre-shift and post-shift levels (mean, median (range)) of selected indicators of renal function (9 weeks in sugarcane cutters and 5 months in referents) in those individuals (23 sugarcane cutters and 25 referents) who took part in both examinations. P-values (mixed effect model) for changes between testing days and for differences between groups are given.

	Cutters, N=23		P for change Cut3 vs. Cut1	Referents, N=25		P for change Ref2 vs. Ref1	P for group diff Cut1 vs. Ref1	P for group diff Cut3 vs. Ref2
	Cut1	Cut3		Ref1	Ref2			
S-creatinine Pre, mg/dL	0.98, 0.91 (0.65 – 2.4)	1.18, 0.95 (0.69 – 3.6)	0.002	0.83, 0.81 (0.45 – 1.3)	0.88, 0.85 (0.63 – 1.3)	0.02	0.07	0.009
S-creatinine Post	1.06, 0.99 (0.78 – 2.5)	1.26, 1.10 (0.73 – 3.3)	0.002	0.93, 0.86 (0.67 – 1.4)	0.94, 0.90 (0.70 – 1.5)	0.74	0.15	0.003
eGFR Pre, mL/min/1.73 m2	109, 111 (37 – 141)	99, 108 (22 – 134)[a]	0.02	116, 116 (75 – 151)	112, 117 (78 – 138)	0.07	0.35	0.03
S-Urea N Pre, mg/dL	11.3, 10.4 (5.9 – 19)	15.9, 15.9 (6.9 – 46)	< 0.001	10.3, 9.6 (5.2 – 18)	9.7, 9.8 (4.3 – 15)	0.29	0.34	< 0.001
S-Urea N Post	13.0, 12.5 (7.4 – 23)	16.3, 14.3 (7.2 – 34)	0.007	11.8, 11.6 (7.1 – 23)	10.8, 11.0 (6.7 – 15)	0.11	0.38	< 0.001
S-K Pre, mmol/L	4.4, 4.3 (3.6 – 5.3)	4.0, 4.0 (2.8 – 5.3)	0.002	4.4, 4.4 (3.8 – 5.0)	4.1, 4.2 (3.3 – 5.5)	0.01	0.91	0.42
S-K Post	3.9, 4.0 (3.2 – 4.7)	3.8, 3.8 (3.1 – 5.6)	0.44	4.1, 4.1 (3.2 – 5.4)	4.2, 4.3 (3.6 – 5.1)	0.11	0.29	0.007
S-phosphate Pre, mg/dL	4.5, 4.6 (2.9 – 5.6)	4.2, 4.2 (2.6 – 5.3)	0.02	3.5, 3.5 (2.3 – 4.6)	3.3, 3.5 (2.2 – 4.0)	0.24	< 0.001	< 0.001
S-phosphate Post	4.4, 4.4 (3.1 – 4.7)	3.8, 3.9 (2.5 – 5.0)	< 0.001	4.0, 4.0 (2.6 – 5.6)	4.0, 3.8 (2.8 – 5.0)	1.0	0.02	0.22
U-NGAL Pre, μg/gCr	18, 10 (0.2 – 57)	72, 17 (3.8 – 351)	0.003	8.6, 6.4 (0.04 – 23)	23.7, 8.0 (0.3 – 214)	0.02	0.10	0.02
U-NGAL Post	15, 11 (1.0 – 87)	106, 32 (1.7 – 960)	< 0.001	8.4, 4.4 (0.09 – 28)	26.7, 5.6 (0.2 – 230)	0.02	0.06	0.001
U-uric acid Pre, mg/gC	472, 433 (240 – 1160)	647, 498 (160 – 1540)	0.12	367, 368 (46 – 610)	454, 423 (280 – 680)	0.11	0.08	0.18
U-uric acid Post[b]	530, 450 (64 – 950)	725, 550 (330 – 2030)	0.04	369, 354 (8.3 – 700)	463, 450 (223 – 700)	0.01	0.002	0.04

[a] Three individuals, all cutters, had eGFR < 60 mL/min.
[b] 22 cutters and 24 referents.

16% increase in mean serum creatinine, a 40% increase in serum urea N, 10% decrease in estimated GFR and two new cases of reduced eGFR (< 60 mL/min).

While the rise in post-shift serum creatinine could reflect dehydration with a loss of extracellular volume (prerenal dysfunction), this is not likely for the pre-shift samples. We cannot exclude a slight increase in muscle mass over the nine-week period, but their mean body weight in fact decreased. The substantial increase in pre-shift NGAL also supports a deleterious effect of sugarcane work on kidney function. Therefore, the rise in serum creatinine and the decrease of eGFR in pre-shift samples likely reflect true renal injury, and are less likely to reflect alterations in hydration status, diet, or changes in muscle mass.

There were also some changes in markers of kidney function in the reference group, although more modest. We have no obvious explanation for the increase in serum creatinine in the referents over 5 months, but one possibility is that there is a seasonal effect on serum creatinine in this region with slightly higher levels when ambient temperature is higher, as has been suggested in some previous studies (Dalpino et al., 2005; Masugata et al., 2011). The mean body weight increased (mean 1.6 kg) in the referents, but it is not likely that this reflected any increase of muscle mass. Their serum urea N did not change but NGAL increased, but less than in the cane cutters.

We are aware of only two longitudinal studies, with a follow-up of workers over a harvest season, in Brazil (Paula Santos et al., 2015) and in Nicaragua (Laws et al., 2015a, 2015b). The Brazilian study showed no increase in serum creatinine in 28 cane cutters over an eight months harvest season, while the study of 51 cane cutters in Nicaragua found a mean increase in pre-shift serum creatinine of 0.07 mg/dL (8%), and a drop in eGFR of 3 mL/min over five months (Laws et al., 2015a). In a larger combined group of sugarcane field workers the decrease in eGFR was significantly larger than in a group of non-field workers. This study also found that NGAL, IL-8, and NAG increased more over the harvest season in field workers compared to non-field workers (Laws et al., 2015b). Our results show the same general pattern as in the study by Laws et al., but the increase in serum creatinine and the decrease in eGFR were larger in the present study in spite of a shorter follow-up period. Hsp72 has been suggested to be a good biomarker for predicting and detecting acute kidney injury (Morales-Buenrostro et al., 2014). In an experimental rat model of AKI, Hsp72 is a reliable biomarker for stratifying different degrees of tubular injury and recovery, as well as for monitoring a renoprotective intervention (Barrera-Chimal et al., 2011), and kidney levels of Hsp72 increase in mice exposed to heat (Islam et al., 2013). Therefore, the tendency towards a cross-shift increase of Hsp72 is biologically plausible, but the evaluation of this biomarker in the present study is hampered by the limited number of sugarcane cutters with detectable Hsp72 levels.

Another interesting result in the present study was the fact that pre-shift serum creatinine seemed to increase (although not statistically significant) already in the first six days of the harvest work. This could possibly indicate that this is a sensitive period, before the workers have been acclimatized to the hard work in a hot environment. In line with this, the cross-shift increase of serum creatinine was largest on the first day, when there was also a significant weight loss, and the cross-shift increase in serum creatinine could reflect a loss of extracellular volume (prerenal dysfunction). The significant increase in KIM-1 in the first week may support this hypothesis, although we cannot explain why KIM-1 returned to normal after 9 weeks.

The aim was to select young healthy men for the study, and therefore individuals with serum creatinine ≥ 1.0 mg/dL at a screening session were excluded. In spite of this, shortly after the screening, at the baseline sampling, several individuals had serum

creatinine higher than the screening cut-off value. The reason for this may be the fact that the analyses were performed at two different laboratories, and/or temporal variability in serum creatinine levels.

The cross-shift decrease of serum potassium in the cutters was of the same size as recently found in the aforementioned study in El Salvador (García-Trabanino et al., 2015), and is likely the result of activation of the renin-angiotensin-aldosterone system (RAAS), which increases the excretion of potassium. It is unclear why the cane cutters had higher pre-shift (but not post-shift) serum phosphate than the referents. Possibly dietary habits differ over time and between cane cutters and referents.

Cross-shift changes of serum creatinine and serum urea N were in agreement with a recent larger study of 189 sugarcane workers in El Salvador (García-Trabanino et al., 2015). In that study there were also cross-shift changes in electrolytes and stronger effects on cardiovascular function. In the study in El Salvador, the cane cutters were examined in the field, immediately after shift. In the present study the post-shift examination of the cutters was performed in their homes, several hours after the work had ceased. Since the workers had then been able to rest, eat and drink, the present study is less optimal for studying cross-shift changes than the study by García-Trabanino et al. (2015). The significant increase in urinary NGAL during the work-day supports renal injury, although there is a report that a rise in serum NGAL could simply reflect dehydration (Nejat et al., 2012). Also the Brazilian study by Paula Santos et al. (2015) found a substantial cross-shift increase in serum creatinine. They found a cross-shift increase of serum creatinine >0.3 mg/dL in 5 out of 28 cane cutters at the end of the harvest season, i.e. compatible with acute kidney injury (AKI) (KDIGO, 2012). In the present study 3 out of 22 cane cutters at mid-harvest had an increase of >0.3 mg/dL. AKI is relatively common in hospitalized patients, often in association with renal ischemia (Bucaloiu et al., 2012). Because it is usually reversible, it was previously not assumed to be a risk factor for chronic kidney disease (CKD). Long-term follow-up of patients with episodes of AKI have, however, shown that these patients run an increased risk of CKD (Bucaloiu et al., 2012). This has also been demonstrated in experimental studies of ischemic AKI in rats (Barrera-Chimal et al., 2011; Rodríguez-Romo et al., in press). Since repeated episodes of AKI seem to occur in sugarcane cutters, it is a reasonable hypothesis that this in the long-term increases the risk of CKD. The association between the cross-shift increase of serum creatinine and the decrease of eGFR over nine weeks in the cutters lends further support to this hypothesis. To the best of our knowledge no previous study examined cross-shift changes and development of kidney function over a longer period. Unfortunately the group size is small, and therefore we cannot exclude that this finding is due to chance. Interestingly, already in 1970, a small case series of South African miners with AKI from heat stroke showed development of CKD with interstitial fibrosis and tubular atrophy at follow-up after 8–21 months (Kew et al., 1970).

Recently elevated urinary concentrations of uric acid have been proposed to contribute to the development of MeN, either through direct effects of soluble uric acid or due to actions of urate crystals on tubular epithelium (Roncal-Jimenez et al., 2015, 2016). The present study confirmed increases of urinary uric acid levels over the working day in the sugarcane cutters (Table 2A), while this was not the case in the reference group (Table 2B). Interestingly, in the seven cutters with urine samples collected post-shift during very hot days in May at the end of the harvest the urinary uric acid levels were very high, and much higher than in the referents sampled in May. This might be due to relatively greater dehydration at that time.

A main limitation of the present study is the modest number of subjects. Other limitations include different timing of the follow-up between the two groups, with the follow-up of the sugarcane cutters at 9 weeks (in January 2013), while the follow-up of the referents occurred at the end of harvest in May 2013. While the study was supposed to include a follow-up in May for the sugarcane workers, 50% of the enrolled cane cutters were fired at the middle of the harvest because they were participating in the study. Second, we also had to collect blood and urine samples from cane cutters at their home instead of examining them on site immediately after they ended the work-shift. This allowed the workers to rehydrate themselves if they had become dehydrated in the field. A third limitation is the fact that the cane cutters had on average a lower socioeconomic status than the reference group. They had fewer years of schooling than the referents and they were shorter and had a lower body weight. These differences at baseline should, however, not be important for the analyses of cross-shift or longitudinal changes. A final limitation is that analyses were performed on frozen samples, and long-term storage can decrease concentrations of KIM-1 and NGAL (Nauta et al., 2012). Despite these limitations, the longitudinal increase in serum creatinine, serum urea N, and urinary NGAL resulted in substantial and statistically significant differences between cane cutters and referents at follow-up. In addition, there were group differences in serum levels of potassium and phosphate. Post-shift urinary uric acid levels were higher in the cane cutters both at start of harvest and at follow-up.

An explanation is warranted about the situation surrounding the firing of the study participants in this region where there are practically no alternative employment opportunities. The workers were not fired directly by the sugar company; the task was delegated to the subcontractors who had hired the workers. The Nicaraguan partners of our research team, with support from the authorities of their university, immediately demanded that the company reinstate these workers, but without success. The principal investigator of a research group working with the company flew down to Nicaragua to mediate with the company. The workers were rehired six weeks later, during which time the project provided them with financial support. The fired workers were not willing to continue their participation in the study. Most of the other workers followed suit. Protecting the identity of workers who participate in a study remains a major concern in planning and executing studies in this region. In a broader context, mounting scientific evidence of a link between sugarcane cutting and Mesoamerican nephropathy, as well as press attention to working conditions and labor rights, is impacting the company's attitude towards improvements.

In conclusion, we found a remarkable decrease of glomerular kidney function, after only 9 weeks of harvest. If glomerular filtration decreases by 10% only after half a harvest season, it is not surprising that chronic kidney disease with severely reduced glomerular filtration is common in this sugarcane area. The cross-shift increase in serum creatinine is probably caused by dehydration (pre-renal dysfunction), which when repeated on a daily basis may cause permanently reduced GFR, and the present study provides some support for this hypothesis. There is a strong need for preventive measures, including the provision of water, rest, and shade.

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Ethics review

All participants signed a written informed consent to participate in the study, which was approved by the IRB at National Autonomous University of Nicaragua, León and the Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán, Mexico.

Disclosures

The authors disclose no conflicts of interest related to this study. Dr Johnson does have patents and patent applications related to blocking fructose and uric acid metabolism, is on the Scientific Board for Amway and XORT therapeutics, has lectured at Danone symposia, and is a member of Colorado Research Partners that is developing inhibitors for fructose metabolism. Dr. Bobadilla does have patents and patent applications related to Hsp72 as a biomarker of acute kidney injury.

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References

Barrera-Chimal, J., Pérez-Villalva, R., Cortés-González, C., et al., 2011. Hsp72 is an early and sensitive biomarker to detect acute kidney injury. EMBO Mol. Med. 3 (1), 5–20.
Brooks, D.R., Ramirez-Rubio, O., Amador, J.J., 2012. CKD in Central America: a hot issue. Am. J. Kidney Dis. 59 (4), 481–484.
Bucaloiu, I.D., Kirchner, H.L., Norfolk, E.R., Hartle, J.E.2nd, Perkins, R.M., 2012. Increased risk of death and de novo chronic kidney disease following reversible acute kidney injury. Kidney Int. 81, 477–485.
Correa-Rotter, R., Wesseling, C., Johnson, R.J., 2014. CKD of unknown origin in Central America: the case for a Mesoamerican nephropathy. Am. J. Kidney Dis. 63 (3), 506–520.
Crowe, J., Nilsson, M., Kjellstrom, T., Wesseling, C., 2015. Heat-related symptoms in sugarcane harvesters. Am. J. Ind. Med. 58 (5), 541–548.
Dalpino, F., Menna-Barreto, L., Castilho, L., de Faria, E., 2005. Biological rhythms of biochemical serum parameters in a Brazilian population: a three-year study. Chronobiol. Int. 22 (5), 925–935.
García-Trabanino, R., Jarquín, E., Wesseling, C., et al., 2015. Heat stress, dehydration, and kidney function in sugarcane cutters in El Salvador – a cross-shift study of workers at risk of Mesoamerican nephropathy. Environ. Res. 142, 746–755.
Herrera, R., Orantes, C.M., Almaguer, M., et al., 2014. Clinical characteristics of chronic kidney disease of nontraditional causes in Salvadoran farming communities. MEDICC Rev. 16 (2), 39–48.
Islam, A., Abraham, P., Hapner, C.D., et al., 2013. Heat exposure induces tissue stress in heat-intolerant, but not heat-tolerant, mice. Stress 16 (2), 244–253.
Johnson, R.J., 2015. Why focus on uric acid? Curr. Med. Res. Opin. 31 (Suppl. 2), S3–S7.
KDIGO, 2012. Kidney disease: improving global outcomes (KDIGO). Acute kidney injury work group. KDIGO 2012 clinical practice guideline for acute kidney injury. Kidney Int. Suppl. 2 (1), 1–138.
Kew, M.C., Abrahams, C., Seftel, H.C., 1970. Chronic interstitial nephritis as a consequence of heat stroke. Q. J. Med. 39, 189–199.
Knochel, J.P., Dotin, L.N., Hamburger, R.J., 1974. Heat stress, exercise, and muscle injury: effects on urate metabolism and renal function. Ann. Intern. Med. 81 (3), 321–328.
Laws, R.L., Brooks, D.R., Amador, J.J., et al., 2015a. Changes in kidney function among Nicaraguan sugarcane workers. Int. J. Occup. Environ. Health 21 (3), 241–250.
Laws, R.L., Brooks, D.R., Amador, J.J., et al., 2015b. Biomarkers of kidney injury among Nicaraguan sugarcane workers. Am. J. Kidney Dis. [E-Pub]
Levey, A.S., Stevens, L.A., Schmid, C.H., et al., 2009. A new equation to estimate glomerular filtration rate. Ann. Intern. Med. 150 (9), 604–612.
López-Marín, L., Chávez, Y., García, X.A., et al., 2014. Histopathology of chronic kidney disease of unknown etiology in Salvadoran agricultural communities. MEDICC Rev. 16 (2), 49–54.
Masugata, H., Senda, S., Inukai, M., et al., 2011. Seasonal variation in estimated glomerular filtration rate based on serum creatinine levels in hypertensive patients. Tohoku J. Exp. Med. 224 (2), 137–142.
Morales-Buenrostro, L.E., Salas-Nolasco, O.I., Barrera-Chimal, J., et al., 2014. Hsp72 is a novel biomarker to predict acute kidney injury in critically ill patients. PLoS One 9 (10), e109407.
Nauta, F.L., Bakker, S.J., Lambers Heerspink, H., et al., 2012. Effect of frozen storage on urinary concentration of kidney damage markers. Am. J. Kidney Dis. 59 (4), 586–589.
Nejat, M., Pickering, J.W., Devarajan, P., et al., 2012. Some biomarkers of acute kidney injury are increased in pre-renal acute injury. Kidney Int. 81 (12), 1254–1262.
Paula Santos, U., Zanetta, D.M., Terra-Filho, M., Burdmann, E.A., 2015. Burnt sugarcance harvesting is associated with acute renal dysfunction. Kidney Int. 87 (4), 792–799.
Peraza, S., Wesseling, C., Aragón, A., et al., 2012. Decreased kidney function among agricultural workers in El Salvador. Am. J. Kidney Dis. 59 (4), 531–540.
Ramírez-Rubio, O., Amador, J.J., Kaufman, J.S., et al., 2015. Urine biomarkers of kidney injury among adolescents in Nicaragua, a region affected by an epidemic of chronic kidney disease of unknown aetiology. Nephrol. Dial. Transplant. [E-Pub].
Rodríguez-Romo, R., Benítez, K., Barrera-Chimal, J., et al., 2016. AT1 receptor antagonism before ischemia prevents the transition of acute kidney injury to chronic kidney disease. Kidney Int., http://dx.doi.org/10.1038/ki.2015.320, in press.
Roncal Jimenez, C.A., Ishimoto, T., Lanaspa, M.A., et al., 2014. Fructokinase activity mediates dehydration-induced renal injury. Kidney Int. 86 (2), 294–302.
Roncal-Jimenez, C., Lanaspa, M.A., Jensen, T., Sanchez-Lozada, L.G., Johnson, R.J., 2015. Mechanisms by which dehydration may lead to chronic kidney disease. Ann. Nutr. Metab. 66 (Suppl. 3), S10–S13.
Roncal-Jimenez, C., García-Trabanino, R., Barregard, L., et al., 2016. Heat stress nephropathy from exercise-induced uric acid crystalluria: a perspective on Mesoamerican nephropathy. Am. J. Kidney Dis. 67, 20–30.
Wesseling, C., Crowe, J., Hogstedt, C., et al., 2014. Resolving the enigma of the Mesoamerican nephropathy: a research workshop summary. Am. J. Kidney Dis. 63 (3), 396–404.
Wijkstrom, J., Leiva, R., Elinder, C.G., et al., 2013. Clinical and pathological characterization of Mesoamerican nephropathy: a new kidney disease in Central America. Am. J. Kidney Dis. 62 (5), 908–918.

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Perspective

Heat Stress Nephropathy From Exercise-Induced Uric Acid Crystalluria: A Perspective on Mesoamerican Nephropathy

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Mesoamerican nephropathy (MeN), an epidemic in Central America, is a chronic kidney disease of unknown cause. In this article, we argue that MeN may be a uric acid disorder. Individuals at risk for developing the disease are primarily male workers exposed to heat stress and physical exertion that predisposes to recurrent water and volume depletion, often accompanied by urinary concentration and acidification. Uric acid is generated during heat stress, in part consequent to nucleotide release from muscles. We hypothesize that working in the sugarcane fields may result in cyclic uricosuria in which uric acid concentrations exceed solubility, leading to the formation of dihydrate urate crystals and local injury. Consistent with this hypothesis, we present pilot data documenting the common presence of urate crystals in the urine of sugarcane workers from El Salvador. High end-of-workday urinary uric acid concentrations were common in a pilot study, particularly if urine pH was corrected to 7. Hyperuricemia may induce glomerular hypertension, whereas the increased urinary uric acid may directly injure renal tubules. Thus, MeN may result from exercise and heat stress associated with dehydration-induced hyperuricemia and uricosuria. Increased hydration with water and salt, urinary alkalinization, reduction in sugary beverage intake, and inhibitors of uric acid synthesis should be tested for disease prevention.
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INDEX WORDS: Mesoamerican nephropathy (MeN); heat stress nephropathy; uric acid crystalluria; etiology; chronic kidney disease (CKD); rhabdomyolysis; uric acid disorder; uricosuria; hyperuricemia; urinary acidification; sugarcane workers; physical exertion; dehydration; Central America; tubular injury; hypothesis.

An epidemic of chronic kidney disease (CKD) of unknown cause is occurring along the Pacific coast of Central America. Although it was first described in 2002,[1] the disease has likely been present for decades.[2] The epidemic, known as Mesoamerican nephropathy (MeN), is observed primarily in men who are working manually in the sugarcane fields in the hotter lower altitudes along the Pacific coast.[3,4] However, MeN also has been reported among farmers of other crops (eg, cotton, corn, and beans), miners, and fishermen, as well as construction, port, and transportation workers living in the same region.[5-8] Individuals typically are asymptomatic,[9] but have an elevated serum creatinine level with absent or minimal proteinuria.[10] Kidney biopsy, when performed, reveals chronic tubulointerstitial disease, often with glomerulosclerosis and evidence of kidney ischemia.[11] Progression to end-stage renal disease is common, and mortality is high due to the scarcity of dialysis therapy. An estimated 20,000 people have died from the epidemic.[12] A variety of potential causes have been proposed, including exposure to

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nephrotoxic pesticides and agrochemicals, use of nonsteroidal anti-inflammatory agents, heavy metal exposure, leptospirosis, and chronic recurrent dehydration (the most favored hypothesis).[3,4]

Could MeN Be a Uric Acid Disorder?

In 1974, Knochel et al[13] suggested that hyperuricemia and uricosuria might have an etiologic role in the condition of "heat stress" nephropathy. Serum uric acid levels commonly increase after exercise in hot conditions, such as from marathon running and off-road motocross, often in association with acute kidney injury (AKI).[14-17] Despite such studies, the pathologic mechanism has not been further explored. Here, we revisit the hypothesis that hyperuricemia and cyclical uricosuria associated with volume depletion (salt loss) and dehydration (water loss)[18] have a contributory causal role in MeN. Specifically, we hypothesize that MeN is initiated by the combination of heat, exercise, and recurrent dehydration (Fig 1).

Daily Dehydration and Volume Depletion

People who work in sugarcane fields are exposed to significant heat that tends to exceed recommended work practices made by the US Occupational Safety and Health Administration.[19,20] Although some sugarcane workers drink as much as 1 to 2 L per hour while they work, they have been shown to develop a modest elevation in serum osmolarity during the day, as well as reduced urine volumes with elevated urinary osmolarity and high urine specific gravity consistent with a water-depleted state.[21,22] While it is likely that workers experience both sodium and water loss, the latter predominates because sweat is hypotonic. Thus, individuals working in the sugarcane fields develop signs of dehydration on a daily basis.

Subclinical Rhabdomyolysis

Exercise in the heat can result in mild muscle injury together with an increase in blood levels of creatine kinase—features consistent with subclinical rhabdomyolysis—in association with biomarkers of kidney damage and decreased kidney function.[14,16,23] The heat component has been shown to be an important factor in increasing the susceptibility to rhabdomyolysis.[23] Intense exercise in the heat of sugarcane fields has been reported to result in mild muscle injury, with a doubling of creatine kinase level.[24] Thus, individuals working in sugarcane fields are at increased risk for muscle injury that is not uncommonly associated with subclinical rhabdomyolysis.

Hyperuricemia

Although subclinical rhabdomyolysis is thought to carry relatively minimal risk for the development of decreased kidney function, it releases substrate (nucleic acids) from the damaged muscle that could

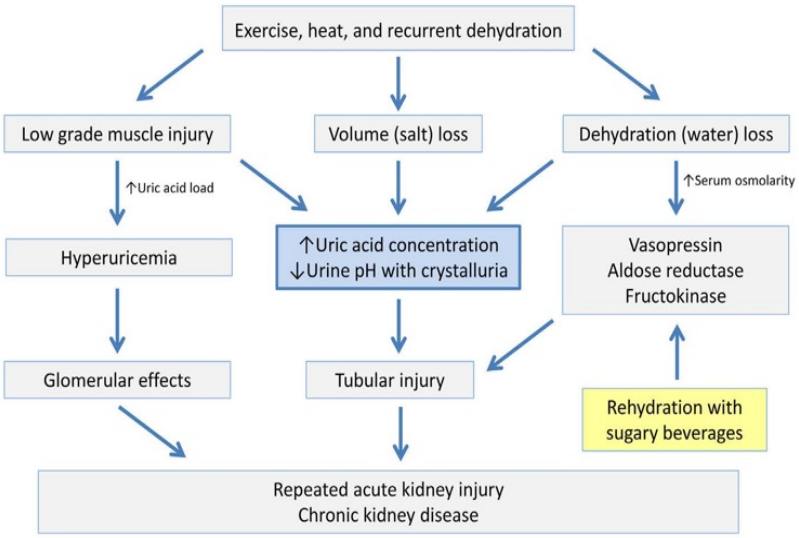


Figure 1. Proposed mechanism for Mesoamerican nephropathy. Exercising under hot conditions with inadequate hydration results in both dehydration (water loss), with an increase in serum osmolarity, and salt loss (volume depletion). An increase in serum uric acid levels occurs from subclinical muscle injury (increased substrate release) and water and salt depletion (increased reabsorption). Volume depletion concentrates the urine, while acid load (ie, lactate) and the effects of aldosterone on the kidney acidify it. As the workday proceeds, uric acid concentrations exceed their solubility due to both high concentrations and urine acidity. Serum uric acid results in glomerular hypertension, while urinary uric acid injures tubules through crystalline and noncrystalline effects. Low-grade proximal tubular injury also occurs from an osmolarity-induced increase in circulating vasopressin and activation of aldose reductase and fructokinase in the proximal tubule, the latter of which may be amplified by drinking fructose-containing soft drinks or other sugary beverages. Kidney injury occurs and is amplified on a daily basis with recurrent exposure to heat, exercise, and dehydration. Over time, chronic kidney disease develops.

increase uric acid generation.[13] Serum uric acid levels also increase in the setting of volume depletion and a decreased kidney perfusion.[13] Hyperuricemia has been reported to be common in MeN.[11,20] For example, in a recent study of 189 sugar cane workers in El Salvador, we showed that workers' mean serum uric acid levels were 6.5 mg/dL in the morning and 7.2 mg/dL in the afternoon.[20] Moreover, 21 of 23 individuals in this study with estimated glomerular filtration rates < 60 mL/min/1.73 m^2 had hyperuricemia. Thus, hyperuricemia is common in sugarcane workers and often worsens during the course of the workday.

Osmotic Effects on the Kidney

Sweating results in hypotonic fluid losses, increasing the risk for hyperosmolarity and dehydration (water loss). We have previously reported that hyperosmolarity associated with water depletion causes activation of the polyol (aldose reductase)-fructokinase pathway in the kidney, which leads to intrarenal fructose generation that is subsequently metabolized, releasing uric acid as a side product.[25] This pathway not only provides an additional mechanism for uric acid generation, but also has been shown to mediate proximal tubular injury due to fructose-dependent decreases in adenosine triphosphate within the cell and the induction of oxidative stress.[26] We found kidney cortical accumulation of uric acid in an animal model of heat-induced dehydration-associated kidney injury, in association with the development of mild CKD and fibrosis.[25] Thus, recurrent dehydration in itself is capable of causing some proximal tubular damage.

Urinary Acidification

Urine pH in the general population averages between 5.9 and 6.0,[27-29] whereas that in sugarcane workers is commonly more acidic. In a recent study of sugarcane workers in Costa Rica, we found that pH was markedly lower in postshift urine samples: urine pH was ≤5 (by dipstick) in >80% of individuals, and 50% had elevated urinary specific gravity (>1.025).[21] This urinary acidification is likely due to both the release of lactic acid from the muscle (production) and the effects of volume (salt) depletion, which lead to increased proximal absorption of sodium and bicarbonate, as well as distal stimulation of aldosterone. Thus, urinary acidification occurs commonly in sugarcane workers and is likely a consequence of both volume (salt) depletion and lactate generation.

Studies in Sugarcane Workers

In our recent observational study of 189 cane cutters in El Salvador near the end of the 6-month harvest,[20] we examined serum and urine samples before and after work shifts and found that ~20% of workers had preshift serum creatinine levels ≥ 1.2 mg/dL, consistent with some underlying reduction in kidney function. Indicators of dehydration were present, as noted by a significant increase in urine osmolarity during the workday, especially in individuals with normal preshift serum creatinine levels.[20] A blunted increase in urine osmolarity was observed in individuals with elevated preshift serum creatinine levels, which likely reflects the urine concentrating defect known to occur in CKD, but could also be a consequence of the impaired function of the urine concentrating response that has been recognized to exist in the setting of chronic dehydration.[30] Fluid intake was similar between groups (5.2 L in individuals with serum creatinine < 1.2 mg/dL vs 5.0 L in individuals with serum creatinine ≥ 1.2 mg/dL), as were changes in body weight (−0.1 vs +0.3 kg, respectively).

We have now performed a small pilot study that includes 10 male sugarcane workers from the mentioned observational study. Participants for the pilot study were selected based on morning serum creatinine level; we included 5 individuals with an elevated (≥1.2 mg/dL) preshift creatinine concentration (mean age, 38 years; mean number of harvests, 14) and 5 with preshift creatinine levels < 1.2 mg/dL (mean age, 30 years; mean number of harvests, 11). The objective of the pilot study was to look for evidence of cyclic uricosuria; brief methods are provided in Item S1.

In our pilot sample, serum uric acid levels increased similarly during the day in all individuals (Table 1) and reached hyperuricemia (defined as ≥7.0 mg/dL) in all 5 individuals with baseline elevated serum creatinine levels and in 2 of 5 individuals with normal baseline serum creatinine values. This contrasts with the normal pattern in which serum uric acid levels decrease during the afternoon.[31] Urine pH was measured by dipstick in the field and again in the laboratory with a pH meter, using samples that had been frozen and subsequently thawed. Consistent with another study,[21] urine pH was lower in the post- versus preshift urine samples of sugarcane workers; this difference reached significance in samples measured using the pH meter.

Uric acid solubility is strongly influenced by urinary uric acid concentration and pH, with decreasing solubility as urine acidity increases.[32] Interestingly, uric acid concentrations remained in the 20- to 50-mg/dL range in our pilot study (Table 1; of note, we show actual uric acid concentrations because it is not the uric acid–creatinine ratio that is important for solubility, but rather the uric acid concentration itself). Nevertheless, polarized light microscopy of the urinary sediment showed the presence of amorphous and box-shaped dihydrate uric acid crystals that were negatively birefringent in most samples (Fig 2). In

Table 1. Pre- and Postshift Serum and Urine Values From 10 Male Agricultural Workers, by Preshift Scr Level

Preshift Scr	Preshift	Postshift	P[a]
<1.2 mg/dL			
Serum osmolarity, mOsm/L	275 (269-287)	275 (263-292)	0.9
Scr, mg/dL	0.86 (0.80-1.01)	1.03 (0.95-1.22)	0.03
Serum uric acid, mg/dL	5.8 (5.3-6.4)	7.0 (6.3-7.7)	0.007
Urine specific gravity	1.014 (1.010-1.020)	1.028 (1.025-1.030)	<0.001
Urine osmolarity, mOsm/L	399.2 (163-620)	798.6 (382-1031)	0.03
Urine pH			
By dipstick in the field	5.9 (5-7)	5.2 (5-6)	0.2
By pH meter in the lab	6.36 (5.9-6.9)	5.80 (5.5-6.2)	0.03
Urine uric acid, mg/dL			
Before correction to pH 7	30.5 (23-46)	42.4 (29-79)	0.3
After correction to pH 7	34.7 (26-58)	83.5 (55-119)	<0.01
≥1.2 mg/dL			
Serum osmolarity, mOsm/L	275 (268-281)	278 (269-292)	0.6
Scr, mg/dL	1.42 (1.21-1.97)	1.56 (1.22-2.12)	0.5
Serum uric acid, mg/dL	7.5 (6.4-8.8)	8.7 (7.0-10.4)	0.2
Urine specific gravity	1.017 (1.015-1.020)	1.025 (1.015-1.030)	0.06
Urine osmolarity, mOsm/L	656.6 (387-994)	707.2 (505-1045)	0.8
Urine pH			
By dipstick in the field	5.7 (5-6)	5.4 (5-7)	0.6
By pH meter in the lab[b]	6.08 (5.7-6.2)	5.75 (5.6-5.9)	0.04
Urine uric acid, mg/dL			
Before correction to pH 7	25.5 (17-29)	51.4 (25-67)	0.01
After correction to pH 7	75.7 (21-176)	77.6 (26-150)	0.9

Note: Measurements are presented as mean (range).

Abbreviations: lab, laboratory; Scr, serum creatinine.

[a]By paired *t* test, to determine whether urinary uric acid levels change with correction of pH for each of the 2 groups. $P < 0.05$ was considered significant.

[b]After a freeze-thaw cycle. Urine pH can increase slightly with storage, especially if not frozen.[29]

work in progress, we have also documented elevated uric acid levels with pronounced urine sediments in sugarcane workers from Nicaragua (Fig 3).

In the samples from our El Salvadoran pilot study, correction of the urinary pH to 7 with sodium bicarbonate (using a procedure adapted from previously published methods[33,34]) resulted in solubilization of these crystals, causing an increase in urinary uric acid levels (Table 1). The greatest increase in urinary uric acid levels occurred in postshift urine specimens and in the preshift urine samples from individuals with serum creatinine levels ≥ 1.2 mg/dL, often to levels > 80 mg/dL. Compared with individuals having normal kidney function, those with decreased kidney function appeared to have higher uric acid levels (at pH 7) in preshift urine samples, but the differences in this small sample were not significant.

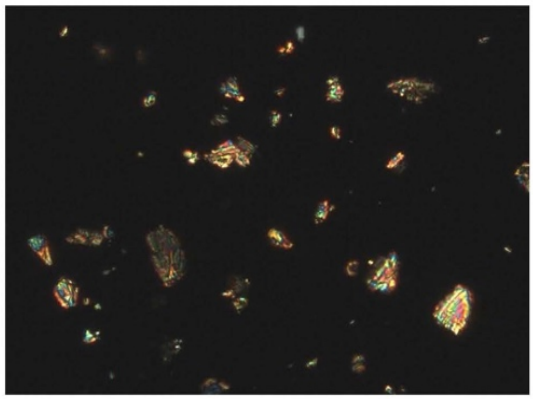
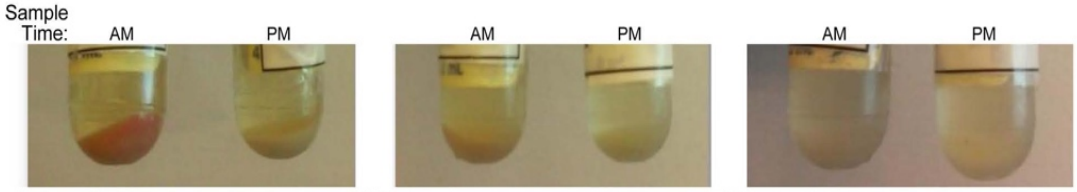


Figure 2. Representative dehydrate urate crystals in a morning urine specimen from an El Salvadoran sugarcane worker participating in the pilot study. Crystals are box-like and negatively birefringent when viewed with crossed circular polarizers (original magnification, ×32).

An important note is that not all individuals develop marked uricosuria, as is evident in the range of values in Table 1. In the original study from which the pilot study participants were drawn, qualitative assessment of urine found urate crystals in just 16% of preshift samples and 22% of postshift samples.[20] Nevertheless, some sugarcane workers are developing uricosuria in which urinary uric acid concentrations exceed solubility due to both high concentrations and urine acidity.

Sugarcane cutters and other workers in Central America often report dysuria that is not due to urinary tract infection; this could be attributable to passing "sandy urine," which may represent early stone formation.[8,35] The condition (known as "chistata" in Nicaragua, "chistate" in Costa Rica, and "mal de orin"



Sample Time	Uric Acid (mg/dL)		
	Supernatant	Sediment	Total
AM	54.2	36.7	90.8
PM	66.8	18.4	85.2

Sample Time	Uric Acid (mg/dL)		
	Supernatant	Sediment	Total
AM	88.2	43.7	131.9
PM	106.2	22.7	128.9

Sample Time	Uric Acid (mg/dL)		
	Supernatant	Sediment	Total
AM	37.6	2.6	40.2
PM	53.7	6.9	60.6

Figure 3. Urine sediment and uric acid concentrations in sugarcane workers in Nicaragua. Pre- and postshift urine sediments from 3 sugarcane workers from an epidemiologic study (C.W., manuscript in preparation). Urine uric acid was measured in both the supernatant and pellet of centrifuged samples (pellets were solubilized with phosphate-buffered saline, pH 7.2). Uric acid levels varied greatly, with 1 individual showing excessively high levels (130 mg/dL), while another showed normal levels (40-60 mg/dL).

in El Salvador) is significantly more frequent among sugarcane cutters than other workers with less or no heat exposure and is associated with other symptoms of volume depletion (lightheadedness, headache, and tachycardia).[36]

Thus, on the basis of the available evidence and our pilot study, we conclude that crystalluria is likely common among sugarcane workers.

Urate Crystalluria as a Mechanism of Tubular Injury

In addition to the potential proinflammatory effects of urate crystals on kidney tubules, high concentrations of noncrystalline uric acid induce inflammation and phenotypic transition of the tubular cells, resulting in tubular injury and fibrosis.[26,37-40] Thus, uricosuria and uric acid crystalluria could provide a mechanism for the predominant tubular injury[11] observed in MeN.

Systemic Hyperuricemia as a Mechanism for Glomerular Injury

Systemic hyperuricemia also increases the risk for CKD due to the development of afferent arteriolar disease altering kidney autoregulation, with increased systemic and glomerular pressure, arteriolar vasoconstriction, a reduction in kidney blood flow, and proinflammatory, pro-oxidative, and vasoactive effects.[41-43]

Other Additive Mechanisms

Volume (salt) depletion, as well as dehydration (water loss), is also a potent stimulus for vasopressin. Dehydration has been reported to accelerate CKD in animals,[44] possibly through effects of vasopressin on glomerular hemodynamics and tubular function.[45-48]

Thus, MeN may represent a type of heat stress nephropathy driven by the effects of serum and urinary uric acid on the kidney coupled with other mechanisms, such as vasopressin release (Fig 1).

Why Is There an Epidemic of MeN Now?

There is evidence that CKD due to MeN has been present for more than 30 years, but that its prevalence has been progressively increasing over recent decades.[12,49] This increase may be related to better awareness and improved diagnosis of the disease; however, additional factors should be considered. The first is global warming, which increases the risk for dehydration.[50] Temperatures have been progressively increasing in El Salvador over the last century, with an average increase of 0.5°C since 1980.[51] Although the overall increase in temperature may appear small, it has been shown that the small average temperature changes associated with global warming are responsible for 75% of the moderate daily hot extremes over land.[52] The second factor that might help explain the recent increase in MeN is that there is more heavy labor, on account of greater sugarcane production throughout the region. Likewise, the productivity demands placed on individual cutters have risen as the sugar sector has become more industrialized. A final factor is the possibility that the type of liquids used for rehydration may play a role in the epidemic. There has been a marked increase in intake of sugary beverages throughout the world.[53,54] Fructose, which is present in soft drinks, can induce tubular injury in laboratory animals.[55] Sugary beverage intake is also associated with increased risk for hyperuricemia and nephrolithiasis[56,57] and may increase urine acidity due to fructose-dependent stimulation of the proximal tubule sodium/hydrogen exchanger.[58]

Relevance to Other Kidney Diseases

Acute Kidney Injury

Cyclic uricosuria may be also relevant to other types of AKI. Table 2 shows several types of AKI in which hyperuricemia and/or uricosuria are common.[14,43,59-79] An increase in serum and urinary uric acid values with crystal formation can cause tumor lysis

Table 2. Possible Role for Hyperuricemia and Uricosuria in Other AKI Syndromes

Condition	Evidence for Role of Uric Acid		Associated With Uricosuria	Evidence That Allopurinol Can Prevent[b]
	Epidemiologic	Experimental[a]		
Tumor lysis–associated AKI	Y[61]	Y[62]	Y[61]	Y[63]
Hypouricemia with AKI	Y[64]		Y[64]	Y[65]
Rhabdomyolysis				
Seizure induced	Y[38,39]			
Marathon induced	Y[14]			
Snake bite induced			Y[66]	
Statin induced			Y[67]	
Radiocontrast	Y[69,70]		Y[72-74,76]	Y[75,116]
Cisplatinum AKI	Y[68]	Y[43]		
Post-CV surgery AKI	Y[41,71,78,79]			

Abbreviations: AKI, acute kidney injury; CV, cardiovascular.
[a]That is, in laboratory animals.
[b]From clinical studies.

syndrome–associated AKI.[80] In addition, there is a condition of hypouricemia and chronic uricosuria due to mutations in the genes encoding the proximal tubule urate transporters (*SLC2A9* and *SLC22A12*).[81-84] Individuals with this condition are asymptomatic until they exercise; upon exertion, they have an increased risk for AKI. It is possible that in the setting of exercise, volume depletion and urinary acidification occur, resulting in uricosuria that exceeds its solubility, resulting in the development of AKI.[64] Consistent with this idea is the observation that AKI in these patients can be prevented with allopurinol treatment, even though they have baseline hypouricemia.[65]

Rhabdomyolysis is also associated with marked hyperuricemia and uricosuria.[85] To our knowledge, studies investigating whether lowering uric acid levels is beneficial for individuals with rhabdomyolysis have not been performed. Nevertheless, the use of allopurinol as adjunctive therapy to lower uric acid levels has been recommended.[85] Rhabdomyolysis is also associated with lactic acid generation and volume depletion, both conditions in which urinary acidification occurs, which might predispose to urate crystalluria formation. Interestingly, the genetic disorder McArdle disease is associated with exercise-induced rhabdomyolysis, yet AKI rarely develops as a result. We speculate that this is because rhabdomyolysis in McArdle disease is not associated with lactate production, making it one of the few types of rhabdomyolysis in which urine pH is usually alkaline (hence leading to urate solubility).

Radiocontrast administration also carries an increased risk for AKI in hyperuricemic individuals[69,70] and is associated with marked uricosuria.[72-74,76] Two controlled trials have reported that prophylactic allopurinol therapy can markedly prevent contrast-associated AKI,[75,77] but additional confirmation is needed. AKI following cardiovascular surgery may also involve this pathway.[41,42] We have reported that hyperuricemia is a major risk factor for AKI and confers greater risk than baseline decreased kidney function.[78] Bicarbonate therapy has also been reported to be protective against the development of post-surgical AKI in a pilot clinical study,[86] but not in a larger follow-up study.[87] However, in the larger study, the bicarbonate solution was concentrated and hypertonic and resulted in some hypernatremia and systemic alkalosis, thus complicating the interpretation of these findings.[88,89]

Chronic Kidney Disease

Hyperuricemia and uricosuria may also be potential modifiable risk factors for CKD. There is already strong evidence from experimental, epidemiologic, and pilot clinical studies that hyperuricemia is an independent risk factor for CKD, that experimental hyperuricemia can both cause and accelerate CKD, and that lowering uric acid levels may slow kidney disease progression in humans (reviewed in[90-93]). Experimentally, the lowering of serum uric acid levels protects CKD by reducing systemic and glomerular hypertension,[94] and consistent with this observation, reducing serum uric acid levels with either an xanthine oxidase inhibitor or a uricosuric agent can block glomerular injury.[93,95] However, what is striking is that in one study in an experimental rat model of CKD,[93] the uricosuric agent provided no protection against the tubulointerstitial injury, whereas the xanthine oxidase inhibitor was protective. This is consistent with the glomerular injury being mediated by hyperuricemia,[94,96] whereas the tubular injury may be attributable more to the urinary uric acid effects.

This hypothesis could provide a mechanistic explanation for why dehydration and low urinary volumes may be risk factors for CKD progression,[97,98] because dehydration would lead to urinary concentration and

acidification. In a longitudinal study, low (<5.5) versus higher (6) urine pH has been reported to increase the risk for CKD.[99] A recent study in healthy Japanese individuals found that 4.4% have acidic urine containing sizeable uric acid crystal–rich sediments.[100] There also is increasing evidence that bicarbonate treatment slows the progression of CKD.[101-103] It was postulated that bicarbonate therapy blocks the effects of systemic metabolic acidosis, but some studies show its benefit occurs even when serum bicarbonate level is normal.[102] One possibility is that the bicarbonate therapy slows the progression of kidney disease by alkalinizing the urine. Factors known to accelerate CKD, such as high-protein diets, acidify the urine, whereas agents that slow kidney disease progression (eg, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and aldosterone antagonists) tend to alkalinize the urine; this suggests that the benefit of these latter treatments could be due in part to urine alkalization.

The hypothesis could also resolve a paradox. Multiple studies show that hyperuricemia is a potent independent predictor for the development of CKD (reviewed in[90]), yet a genetic study[104] suggested the opposite; namely, that elevated serum uric acid levels may protect individuals from CKD. Of note, the genetic study was based on a polymorphism in the *SLC2A9*-encoded urate transporter, which increases serum uric acid levels by increasing reabsorption of urinary uric acid, thereby reducing urinary uric acid at the expense of higher serum uric acid levels. Hence, this polymorphism would uncouple the normal relationship of serum and urine uric acid. Although it might not protect against the glomerular effects of hyperuricemia, it could potentially reduce the risk for tubulointerstitial disease by decreasing uricosuria.

Dehydration and heat stress nephropathy may involve CKD in other regions of the world as well, such as among workers in rice paddies in Northern Sri Lanka.[105,106] CKD also is more common in Pacific Island people, who live in hot climates, tend to drink excessive amounts of soft drinks, and have high serum uric acid levels due to genetic and nongenetic causes.[107] Further, heat stress has been reported as a risk factor for CKD in Thailand.[108] The high frequency of CKD among the Tiwi (Australian aborigine living in hot remote areas of the country)[109] may relate in part to the effects of climate coupled with high sugary beverage intake. Similarly, the epidemic of CKD in El Salvador is more common among sugarcane workers living at hotter low altitudes compared with those working the sugarcane fields at cooler higher altitudes.[7]

Finally, it should be noted that other classes of animals that lack uricase, such as reptiles and birds, are very susceptible to dehydration-associated kidney failure due to increases in serum and urine uric acid levels.[110,111]

Limitations of the Hyperuricemia-Uricosuria Hypothesis

Although we show that uricosuria and urate crystalluria are not uncommon in sugarcane workers, there are nevertheless individuals who do not develop crystalluria. A key epidemiologic question is whether those who develop significant crystalluria are the same as those who develop CKD. However, answering this question will likely require longitudinal studies.

Likewise, it will be important to determine whether the development of kidney disease in this population may be predicted by the presence of chistata, an elevated serum uric acid level, or a low urinary pH. We also recognize that other mechanisms may operate in MeN, and that the hyperuricemia-uricosuria pathway is a hypothesis. Nevertheless, the experimental and epidemiologic evidence for the role of uric acid in kidney disease continues to mount. Our hypothesis is consistent with these data, and the preliminary data presented here provide a new area of investigation for studies aimed at the prevention and treatment of kidney disease.

Proposal for Action

We present the hypothesis that MeN is a uric acid disorder. We propose that uric acid microcrystals (mainly sodium urate) develop when urinary uric acid concentrations are high and that these crystals may drive tubular injury, eventually causing the lesions observed in MeN. Elevated serum uric acid levels may also have a role in inducing some glomerulosclerosis.[93,112] A key action item is to improve the work conditions and hydration practices among the Central American sugarcane workers at risk for CKD. Adequate hydration with water and/or water with electrolytes has been reported to prevent increases in serum uric acid levels that occur with heat and exercise.[113] We also recommend studies to determine whether the mechanism for CKD involves proximal tubular injury with recurrent cyclic exercise-induced uricosuria. For example, if urate crystals are involved in the disease process, a clinical trial of alkalinization with sodium bicarbonate may be an effective way to prevent acute and chronic kidney injury. Studies evaluating allopurinol or other xanthine oxidase inhibitors are also suggested.

For the last several decades, the nephrology community has focused on 4 major causes of CKD: diabetes, hypertension, glomerulonephritis, and autosomal dominant polycystic kidney disease. Nevertheless, many people develop CKD relatively early in life and without frank diabetes and with mild hypertension

or normal blood pressure.[114] Moreover, in patients with hypertension and CKD, we are often unsure if their hypertension is a result or cause of their kidney disease. Sadly, current treatments based on blood pressure and glucose control are only partially effective in slowing CKD.[115] We suggest that further studies investigate the role of hyperuricemia, uricosuria, and acidic pH in acute and chronic kidney diseases. We believe this could represent a major mechanism driving kidney disease.

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SUPPLEMENTARY MATERIAL

Item S1: Brief methods for pilot study.

Note: The supplementary material accompanying this article (http://dx.doi.org/10.1053/j.ajkd.2015.08.021) is available at www.ajkd.org

REFERENCES

1. Trabanino RG, Aguilar R, Silva CR, Mercado MO, Merino RL. Nefropatía terminal en pacientes de un hospital de referencia en El Salvador. *Rev Panam Salud Pública*. 2002;12:202-206.

2. Wesseling C, van Wendel de Joode B, Crowe J, et al. Mesoamerican nephropathy: geographical distribution and time trends of chronic kidney disease mortality between 1970 and 2012 in Costa Rica. *Occup Environ Med*. 2015;72:714-721.

3. Weiner DE, McClean MD, Kaufman JS, Brooks DR. The Central American epidemic of CKD. *Clin J Am Soc Nephrol*. 2013;8:504-511.

4. Correa-Rotter R, Wesseling C, Johnson RJ. CKD of unknown origin in Central America: the case for a Mesoamerican nephropathy. *Am J Kidney Dis*. 2014;63:506-520.

5. Torres C, Aragon A, Gonzalez M, et al. Decreased kidney function of unknown cause in Nicaragua: a community-based survey. *Am J Kidney Dis*. 2010;55:485-496.

6. O'Donnell JK, Tobey M, Weiner DE, et al. Prevalence of and risk factors for chronic kidney disease in rural Nicaragua. *Nephrol Dial Transplant*. 2011;26:2798-2805.

7. Peraza S, Wesseling C, Aragon A, et al. Decreased kidney function among agricultural workers in El Salvador. *Am J Kidney Dis*. 2012;59:531-540.

8. McClean MA, Amador JJ, Laws R, et al. *Biological Sampling Report: Investigating Biomarkers of Kidney Injury and Chronic Kidney Disease Among Workers in Western Nicaragua*. Boston, MA: Boston University School of Public Health: Compliance Advisor Ombudman; 2012.

9. Garcia-Trabanino R, Dominguez J, Jansa JM, Oliver A. [Proteinuria and chronic renal failure in the coast of El Salvador: detection with low cost methods and associated factors]. *Nefrologia*. 2005;25:31-38.

10. Laws RL, Brooks DR, Amador JJ, et al. Changes in kidney function among Nicaraguan sugarcane workers. *Int J Occup Environ Health*. 2015;21:241-250.

11. Wijkstrom J, Leiva R, Elinder CG, et al. Clinical and pathological characterization of Mesoamerican nephropathy: a new kidney disease in Central America. *Am J Kidney Dis*. 2013;62:908-918.

12. Ramirez-Rubio O, McClean MD, Amador JJ, Brooks DR. An epidemic of chronic kidney disease in Central America: an overview. *J Epidemiol Community Health*. 2013;67:1-3.

13. Knochel JP, Dotin LN, Hamburger RJ. Heat stress, exercise, and muscle injury: effects on urate metabolism and renal function. *Ann Intern Med*. 1974;81:321-328.

14. Kratz A, Lewandrowski KB, Siegel AJ, et al. Effect of marathon running on hematologic and biochemical laboratory parameters, including cardiac markers. *Am J Clin Pathol*. 2002;118:856-863.

15. Ascensao A, Ferreira R, Marques F, et al. Effect of off-road competitive motocross race on plasma oxidative stress and damage markers. *Br J Sports Med*. 2007;41:101-105.

16. Neviackas JA, Bauer JH. Renal function abnormalities induced by marathon running. *South Med J*. 1981;74:1457-1460.

17. Hart LE, Egier BP, Shimizu AG, Tandan PJ, Sutton JR. Exertional heat stroke: the runner's nemesis. *Can Med Assoc J*. 1980;122:1144-1150.

18. Mange K, Matsuura D, Cizman B, et al. Language guiding therapy: the case of dehydration versus volume depletion. *Ann Intern Med*. 1997;127:848-853.

19. Crowe J, Wesseling C, Solano BR, et al. Heat exposure in sugarcane harvesters in Costa Rica. *Am J Ind Med*. 2013;56:1157-1164.

20. Garcia-Trabanino R, Jarquín E, Wesseling C, et al. Heat stress, dehydration, and kidney function in sugarcane cutters in El Salvador – a cross-shift study of workers at risk of Mesoamerican nephropathy [published online ahead of print July 23, 2015]. *Environ Res*. http://dx.doi.org/10.1016/j.envres.2015.07.007.

21. Crowe J, Nilsson M, Kjellstrom T, et al. 0401 Repeated pre and post-shift urinalyses show kidney dysfunction among Costa Rican sugarcane cutters exposed to heat stress [abstract]. *Occup Environ Med*. 2014;71(suppl 1):A51.

22. Solis G. *Impacto de las medidas preventivas para evitar el deterioro de la función renal por el Síndrome de Golpe por Calor en trabajadores agrícolas del Ingenio San Antonio del Occidente de Nicaragua, Ciclo Agrícola 2005-2006*. León: Universidad Nacional Autonoma de Nicaragua; 2007.

23. Junglee NA, Di Felice U, Dolci A, et al. Exercising in a hot environment with muscle damage: effects on acute kidney injury biomarkers and kidney function. *Am J Physiol Renal Physiol*. 2013;305:F813-F820.

24. Paula Santos U, Zanetta DM, Terra-Filho M, Burdmann EA. Burnt sugarcane harvesting is associated with acute renal dysfunction. *Kidney Int*. 2015;87:792-799.

25. Roncal Jimenez CA, Ishimoto T, Lanaspa MA, et al. Fructokinase activity mediates dehydration-induced renal injury. *Kidney Int*. 2014;86:294-302.

26. Cirillo P, Gersch MS, Mu W, et al. Ketohexokinase-dependent metabolism of fructose induces proinflammatory mediators in proximal tubular cells. *J Am Soc Nephrol*. 2009;20:545-553.

27. Michaud DS, Troiano RP, Subar AF, et al. Comparison of estimated renal net acid excretion from dietary intake and body size with urine pH. *J Am Diet Assoc*. 2003;103:1001-1007; discussion 1007.

28. Welch AA, Mulligan A, Bingham SA, Khaw KT. Urine pH is an indicator of dietary acid-base load, fruit and vegetables and meat intakes: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk population study. *Br J Nutr*. 2008;99:1335-1343.

29. Cook JD, Strauss KA, Caplan YH, Lodico CP, Bush DM. Urine pH: the effects of time and temperature after collection. *J Anal Toxicol*. 2007;31:486-496.

30. Bankir L, Fischer C, Fischer S, et al. Adaptation of the rat kidney to altered water intake and urine concentration. *Pflugers Arch*. 1988;412:42-53.

31. Kanabrocki EL, Third JL, Ryan MD, et al. Circadian relationship of serum uric acid and nitric oxide. *JAMA*. 2000;283:2240-2241.

32. Iwata H, Nishio S, Yokoyama M, Matsumoto A, Takeuchi M. Solubility of uric acid and supersaturation of monosodium urate: why is uric acid so highly soluble in urine? *J Urol*. 1989;142:1095-1098.

33. Haskins HD. The uric acid solvent power of urine after treatment with piperazin, lysidin, lithium carbonate, and other alkalies. *Arch Intern Med*. 1916;17:405-414.

34. Kippen I, Klinenberg JR, Weinberger A, Wilcox WR. Factors affecting urate solubility in vitro. *Ann Rheum Dis*. 1974;33:313-317.

35. Ramirez-Rubio O, Brooks DR, Amador JJ, et al. Chronic kidney disease in Nicaragua: a qualitative analysis of semi-structured interviews with physicians and pharmacists. *BMC Public Health*. 2013;13:350.

36. Crowe J, Nilsson M, Kjellstrom T, Wesseling C. Heat-related symptoms in sugarcane harvesters. *Am J Ind Med*. 2015;58:541-548.

37. Ryu ES, Kim MJ, Shin HS, et al. Uric acid-induced phenotypic transition of renal tubular cells as a novel mechanism of chronic kidney disease. *Am J Physiol Renal Physiol*. 2013;304:F471-F480.

38. Zhou Y, Fang L, Jiang L, et al. Uric acid induces renal inflammation via activating tubular NF-kappaB signaling pathway. *PLoS One*. 2012;7:e39738.

39. Han HJ, Lim MJ, Lee YJ, et al. Uric acid inhibits renal proximal tubule cell proliferation via at least two signaling pathways involving PKC, MAPK, cPLA2, and NF-kappaB. *Am J Physiol Renal Physiol*. 2007;292:F373-F381.

40. Verzola D, Ratto E, Villaggio B, et al. Uric acid promotes apoptosis in human proximal tubule cells by oxidative stress and the activation of NADPH oxidase NOX 4. *PLoS One*. 2014;9:e115210.

41. Ejaz AA, Beaver TM, Shimada M, et al. Uric acid: a novel risk factor for acute kidney injury in high-risk cardiac surgery patients? *Am J Nephrol*. 2009;30:425-429.

42. Ejaz AA, Mu W, Kang DH, et al. Could uric acid have a role in acute renal failure? *Clin J Am Soc Nephrol*. 2007;2:16-21.

43. Roncal CA, Mu W, Croker B, et al. Effect of elevated serum uric acid on cisplatin-induced acute renal failure. *Am J Physiol Renal Physiol*. 2007;292:F116-F122.

44. Bouby N, Bachmann S, Bichet D, Bankir L. Effect of water intake on the progression of chronic renal failure in the 5/6 nephrectomized rat. *Am J Physiol*. 1990;258:F973-F979.

45. Bankir L, Bouby N, Ritz E. Vasopressin: a novel target for the prevention and retardation of kidney disease? *Nat Rev Nephrol*. 2013;9:223-239.

46. Bardoux P, Bichet DG, Martin H, et al. Vasopressin increases urinary albumin excretion in rats and humans: involvement of V2 receptors and the renin-angiotensin system. *Nephrol Dial Transplant*. 2003;18:497-506.

47. Bardoux P, Martin H, Ahloulay M, et al. Vasopressin contributes to hyperfiltration, albuminuria, and renal hypertrophy in diabetes mellitus: study in vasopressin-deficient Brattleboro rats. *Proc Natl Acad Sci U S A*. 1999;96:10397-10402.

48. Johnson RJ, Rodriguez-Iturbe B, Roncal-Jimenez C, et al. Hyperosmolarity drives hypertension and CKD–water and salt revisited. *Nat Rev Nephrol*. 2014;10:415-420.

49. Wesseling C, van Wendel de Joode B, Crowe J, Rittner R, Jakobsson K. 0204 Mesoamerican nephropathy in Costa Rica: geographical distribution and time trends of chronic kidney disease mortality between 1970 and 2012 [abstract]. *Occup Environ Med*. 2014;71(suppl 1):A27.

50. Johnson RJ, Glaser J, Sanchez-Lozada LG. Chronic kidney disease of unknown etiology: a disease related to global warming? *MEDICC Rev*. 2014;16:79-80.

51. Berkeley Earth. Regional climate change: El Salvador. 2015. http://berkeleyearth.lbl.gov/regions/el-salvador. Accessed April 21, 2015.

52. Fischer EM, Knutti R. Anthropogenic contribution to global occurrence of heavy precipitation and high temperature extremes. *Nature Climate Change*. 2015;5:560-565.

53. Anderson TA. Recent trends in carbohydrate consumption. *Annu Rev Nutr*. 1982;2:113-132.

54. Nielsen SJ, Popkin BM. Changes in beverage intake between 1977 and 2001. *Am J Prev Med*. 2004;27:205-210.

55. Nakayama T, Kosugi T, Gersch M, et al. Dietary fructose causes tubulointerstitial injury in the normal rat kidney. *Am J Physiol Renal Physiol*. 2010;298:F712-F720.

56. Choi HK, Curhan G. Soft drinks, fructose consumption, and the risk of gout in men: prospective cohort study. *BMJ*. 2008;336:309-312.

57. Taylor EN, Curhan GC. Fructose consumption and the risk of kidney stones. *Kidney Int*. 2008;73:207-212.

58. Cabral PD, Hong NJ, Hye Khan MA, et al. Fructose stimulates Na/H exchange activity and sensitizes the proximal tubule to angiotensin II. *Hypertension*. 2014;63:e68-e73.

59. Luhdorf K, Petersson H, Pedersen K. Grand mal-provoked hyperuricemia. *Acta Neurol Scand*. 1978;58:280-287.

60. Warren DJ, Leitch AG, Leggett RJ. Hyperuricaemic acute renal failure after epileptic seizures. *Lancet*. 1975;2:385-387.

61. Conger JD. Acute uric acid nephropathy. *Med Clin North Am*. 1990;74:859-871.

62. Conger JD, Falk SA. Intrarenal dynamics in the pathogenesis and prevention of acute urate nephropathy. *J Clin Invest*. 1977;59:786-793.

63. Darmon M, Vincent F, Camous L, et al. Tumour lysis syndrome and acute kidney injury in high-risk haematology patients in the rasburicase era. A prospective multicentre study from the Groupe de Recherche en Reanimation Respiratoire et Onco-Hematologique. *Br J Haematol*. 2013;162:489-497.

64. Ichida K, Hosoyamada M, Hisatome I, et al. Clinical and molecular analysis of patients with renal hypouricemia in Japan - influence of URAT1 gene on urinary urate excretion. *J Am Soc Nephrol*. 2004;15:164-173.

65. Bhasin B, Stiburkova B, De Castro-Pretelt M, et al. Hereditary renal hypouricemia: a new role for allopurinol? *Am J Med*. 2014;127:e3-e4.

66. Frezzatti R, Silveira PF. Allopurinol reduces the lethality associated with acute renal failure induced by Crotalus durissus

terrificus snake venom: comparison with probenecid. *PLoS Negl Trop Dis*. 2011;5:e1312.

67. Bairaktari E, Seferiadis K, Liamis G, et al. Rhabdomyolysis-related renal tubular damage studied by proton nuclear magnetic resonance spectroscopy of urine. *Clin Chem*. 2002;48:1106-1109.

68. Nanji AA, Stewart DJ, Mikhael NZ. Hyperuricemia and hypoalbuminemia predispose to cisplatin-induced nephrotoxicity. *Cancer Chemother Pharmacol*. 1986;17:274-276.

69. Toprak O, Cirit M, Esi E, et al. Hyperuricemia as a risk factor for contrast-induced nephropathy in patients with chronic kidney disease. *Catheter Cardiovasc Interv*. 2006;67:227-235.

70. Liu Y, Tan N, Chen J, et al. The relationship between hyperuricemia and the risk of contrast-induced acute kidney injury after percutaneous coronary intervention in patients with relatively normal serum creatinine. *Clinics (Sao Paulo)*. 2013;68:19-25.

71. Ejaz AA, Kambhampati G, Ejaz NI, et al. Post-operative serum uric acid and acute kidney injury. *J Nephrol*. 2012;25:497-505.

72. McCullough PA. Radiocontrast-induced acute kidney injury. *Nephron Physiol*. 2008;109:61-72.

73. Kelley WN. Uricosuria and x-ray contrast agents. *N Engl J Med*. 1971;284:975-976.

74. Postlethwaite AE, Kelley WN. Uricosuric effect of radiocontrast agents. A study in man of four commonly used preparations. *Ann Intern Med*. 1971;74:845-852.

75. Kumar S, Bhawani G, Kumari N, et al. Comparative study of renal protective effects of allopurinol and *N*-acetyl-cysteine on contrast induced nephropathy in patients undergoing cardiac catheterization. *J Clin Diagn Res*. 2014;8:HC03-HC07.

76. Mudge GH. Uricosuric action of cholecystographic agents. A possible factor in nephrotoxicity. *N Engl J Med*. 1971;284:929-933.

77. Erol T, Tekin A, Katircibasi MT, et al. Efficacy of allopurinol pretreatment for prevention of contrast-induced nephropathy: a randomized controlled trial. *Int J Cardiol*. 2013;167:1396-1399.

78. Lapsia V, Johnson RJ, Dass B, et al. Elevated uric acid increases the risk for acute kidney injury. *Am J Med*. 2012;125.302 e309-e317.

79. Joung KW, Jo JY, Kim WJ, et al. Association of preoperative uric acid and acute kidney injury following cardiovascular surgery. *J Cardiothorac Vasc Anesth*. 2014;28:1440-1447.

80. Ronco C, Inguaggiato P, Bordoni V, et al. Rasburicase therapy in acute hyperuricemia and renal dysfunction. *Contrib Nephrol*. 2005;147:115-123.

81. Kaito H, Ishimori S, Nozu K, et al. Molecular background of urate transporter genes in patients with exercise-induced acute kidney injury. *Am J Nephrol*. 2013;38:316-320.

82. Kikuchi Y, Koga H, Yasutomo Y, et al. Patients with renal hypouricemia with exercise-induced acute renal failure and chronic renal dysfunction. *Clin Nephrol*. 2000;53:467-472.

83. Shen H, Feng C, Jin X, et al. Recurrent exercise-induced acute kidney injury by idiopathic renal hypouricemia with a novel mutation in the *SLC2A9* gene and literature review. *BMC Pediatr*. 2014;14:73.

84. Ishikawa I, Nakagawa M, Hayama S, Yoshida S, Date T. Acute renal failure with severe loin pain and patchy renal ischaemia after anaerobic exercise (ALPE) (exercise-induced acute renal failure) in a father and child with URAT1 mutations beyond the W258X mutation. *Nephrol Dial Transplant*. 2005;20:1015.

85. Vanholder R, Sever MS, Erek E, Lameire N. Rhabdomyolysis. *J Am Soc Nephrol*. 2000;11:1553-1561.

86. Haase M, Haase-Fielitz A, Bellomo R, et al. Sodium bicarbonate to prevent increases in serum creatinine after cardiac surgery: a pilot double-blind, randomized controlled trial. *Crit Care Med*. 2009;37:39-47.

87. Haase M, Haase-Fielitz A, Plass M, et al. Prophylactic perioperative sodium bicarbonate to prevent acute kidney injury following open heart surgery: a multicenter double-blinded randomized controlled trial. *PLoS Med*. 2013;10:e1001426.

88. Wesson DE, Simoni J. Acid retention during kidney failure induces endothelin and aldosterone production which lead to progressive GFR decline, a situation ameliorated by alkali diet. *Kidney Int*. 2010;78:1128-1135.

89. Goraya N, Simoni J, Jo CH, Wesson DE. A comparison of treating metabolic acidosis in CKD stage 4 hypertensive kidney disease with fruits and vegetables or sodium bicarbonate. *Clin J Am Soc Nephrol*. 2013;8:371-381.

90. Johnson RJ, Nakagawa T, Jalal D, et al. Uric acid and chronic kidney disease: which is chasing which? *Nephrol Dial Transplant*. 2013;28:2221-2228.

91. Goicoechea M, de Vinuesa SG, Verdalles U, et al. Effect of allopurinol in chronic kidney disease progression and cardiovascular risk. *Clin J Am Soc Nephrol*. 2010;5:1388-1393.

92. Siu YP, Leung KT, Tong MK, Kwan TH. Use of allopurinol in slowing the progression of renal disease through its ability to lower serum uric acid level. *Am J Kidney Dis*. 2006;47:51-59.

93. Kang DH, Nakagawa T, Feng L, et al. A role for uric acid in the progression of renal disease. *J Am Soc Nephrol*. 2002;13:2888-2897.

94. Sanchez-Lozada LG, Tapia E, Santamaria J, et al. Mild hyperuricemia induces vasoconstriction and maintains glomerular hypertension in normal and remnant kidney rats. *Kidney Int*. 2005;67:237-247.

95. Sanchez-Lozada LG, Tapia E, Soto V, et al. Effect of febuxostat on the progression of renal disease in 5/6 nephrectomy rats with and without hyperuricemia. *Nephron Physiol*. 2008;108:69-78.

96. Sanchez-Lozada LG, Tapia E, Avila-Casado C, et al. Mild hyperuricemia induces glomerular hypertension in normal rats. *Am J Physiol Renal Physiol*. 2002;283:F1105-F1110.

97. Sontrop JM, Dixon SN, Garg AX, et al. Association between water intake, chronic kidney disease, and cardiovascular disease: a cross-sectional analysis of NHANES data. *Am J Nephrol*. 2013;37:434-442.

98. Clark WF, Sontrop JM, Macnab JJ, et al. Urine volume and change in estimated GFR in a community-based cohort study. *Clin J Am Soc Nephrol*. 2011;6:2634-2641.

99. Nakanishi N, Fukui M, Tanaka M, et al. Low urine pH Is a predictor of chronic kidney disease. *Kidney Blood Press Res*. 2012;35:77-81.

100. Ogawa S, Takiguchi J, Nako K, et al. Elucidation of the etiology and characteristics of pink urine in young healthy subjects [published online ahead of print December 5, 2014]. *Clin Exp Nephrol*. http://dx.doi.org/10.1007/s10157-014-1066-y.

101. Abramowitz MK, Melamed ML, Bauer C, Raff AC, Hostetter TH. Effects of oral sodium bicarbonate in patients with CKD. *Clin J Am Soc Nephrol*. 2013;8:714-720.

102. Simon EE, Hamm LL. The role of bicarbonate in CKD: evidence bulks up. *Clin J Am Soc Nephrol*. 2013;8:703-705.

103. Nath KA, Hostetter MK, Hostetter TH. Pathophysiology of chronic tubulo-interstitial disease in rats. Interactions of dietary acid load, ammonia, and complement component C3. *J Clin Invest*. 1985;76:667-675.

104. Hughes K, Flynn T, de Zoysa J, Dalbeth N, Merriman TR. Mendelian randomization analysis associates increased serum urate, due to genetic variation in uric acid transporters, with improved renal function. *Kidney Int*. 2014;85:344-351.

105. Jayatilake N, Mendis S, Maheepala P, Mehta FR. Chronic kidney disease of uncertain aetiology: prevalence and causative factors in a developing country. *BMC Nephrol*. 2013;14:180.

106. Nanayakkara S, Komiya T, Ratnatunga N, et al. Tubulointerstitial damage as the major pathological lesion in endemic chronic kidney disease among farmers in North Central Province of Sri Lanka. *Environ Health Prev Med*. 2012;17:213-221.

107. Johnson RJ, Lanaspa MA, Sanchez-Lozada LG, et al. Fat storage syndrome in Pacific peoples: a combination of environment and genetics? *Public Health Dialogue*. 2014;20:11-16.

108. Tawatsupa B, Lim LL, Kjellstrom T, Seubsman SA, Sleigh A. Association between occupational heat stress and kidney disease among 37,816 workers in the Thai Cohort Study (TCS). *J Epidemiol*. 2012;22:251-260.

109. McDonald SP, Maguire GP, Hoy WE. Renal function and cardiovascular risk markers in a remote Australian aboriginal community. *Nephrol Dial Transplant*. 2003;18:1555-1561.

110. Dutton CJ, Taylor P. A comparison between pre- and posthibernation morphometry, hematology, and blood chemistry in viperid snakes. *J Zoo Wildl Med*. 2003;34:53-58.

111. Lumeij JT. Nephrology. In: Ritchie BW, Harrison GJ, Harrison LR, eds. *Avian Medicine*. Lake Worth, FL: Wingers Publishing Inc; 1994:538-555.

112. Nakagawa T, Mazzali M, Kang DH, et al. Hyperuricemia causes glomerular hypertrophy in the rat. *Am J Nephrol*. 2003;23:2-7.

113. Francis KT. Effect of water and electrolyte replacement during exercise in the heat on biochemical indices of stress and performance. *Aviat Space Environ Med*. 1979;50:115-119.

114. Coresh J, Selvin E, Stevens LA, et al. Prevalence of chronic kidney disease in the United States. *JAMA*. 2007;298:2038-2047.

115. Vilayur E, Harris DC. Emerging therapies for chronic kidney disease: what is their role? *Nat Rev Nephrol*. 2009;5:375-383.

116. Erol T, Tekin A, Katircibasi MT, et al. Efficacy of allopurinol pretreatment for prevention of contrast-induced nephropathy: a randomized controlled trial. *Int J Cardiol*. 2013;167:1396-1399.

Appendix E: A list of conference abstracts

E.T. Smpokou, B. La Rosa Garcia, J. Le Blond, J. Glaser, A. Camacho, D. Faber, A. Aragón, J. Norman1, N. Pearce, D. Nitsch, **M. González-Quiroz,** B. Caplin, J. Morton. Rapid Loss of Kidney Function Amongst Apparently Healthy Young Adults from Communities at Risk of Mesoamerican Nephropathy. Abstract for ICOH 2018. Accepted

Marvin González-Quiroz; Armando Camacho; Dorien Faber; Aurora Aragón; Catharina Wesseling; Jason Glaser; Jennifer Le Blond; Neil Pearce; Dorothea Nitsch; Ben Caplin. Average rate of decline in eGFR is 2.7 mL/min/.173m^2 per year amongst apparently healthy young adults from communities at risk of Mesoamerican nephropathy. Poster presentation in the World Congress of Nephrology in ISN WCN 2017. México D.F, April 2017.

Marvin González-Quiroz; Dorien Faber; Armando Camacho; Ilana Weiss; Alberto Berrios; Aurora Aragón. Knowledge, attitudes and practices of patients and caregivers in a continuous ambulatory peritoneal dialysis program in Nicaragua. Poster presentation in the World Congress of Nephrology in ISN WCN 2017. México D.F, April 2017.

Smpokou ET, La Rosa Garcia B, Morton J, Le Blond J, Camacho A, Faber D, Aragon A, Norman J, Pearce N, Nitsch D, **Gonzalez-Quiroz M**, Caplin B. Baseline urinary findings in young adults with progressive kidney dysfunction in Nicaragua. Abstract for UK Kidney week 2017.

González-Quiroz M, Camacho A, Faber D, Aragón A, Wesseling C, Glaser J, LeBlond J, Smeeth L, Dorothea Nitsch D, Pearce N, Caplin B. Rapid Loss of Kidney Function Amongst Apparently Healthy Young Adults from Communities at Risk of Mesoamerican Nephropathy. Abstract for ASN Kidney Week 2016.

González-Quiroz M, Camacho A, Faber D, Aragón A, Wesseling C, Glaser J, LeBlond J, Smeeth L, Dorothea Nitsch D, Pearce N, Caplin B: Rapid Loss of Kidney Function Amongst Apparently Healthy Young Adults from Communities at High Risk of Mesoamerican Nephropathy: Interim Results from a Community-Based Cohort Study in North West Nicaragua. Abstract for annual UK renal meeting 2016.